PATHOGEN-HOST RELATIONSHIPS BETWEEN *ERYSIPHE CRUCIFERARUM* AND MEMBERS OF THE FAMILY CRUCIFERAE

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Ph.D. Thesis

University of Edinburgh

May 1985

DECLARATION

This is to declare that this thesis has been composed by myself and has not been accepted in any previous application for a degree. The work of which it is a record has been done by myself and all sources of information have specifically been acknowledged by means of references.

James McKenzie Munro

May 1985

ACKNOWLEDGEMENTS

During the past three years I have been indebted to many people without whose generous aid this work would have been considerably more difficult. It is now a pleasure to record my gratitude for their assistance.

Dr J.H. Lennard, my supervisor, for his encouragement and constructive criticism throughout the course of my experimental work and in the preparation of this thesis.

Dr I.H. McNaughton and Dr C.J. Williamson, SCRI, for their helpful discussions and providing seed samples.

Members of the Applied Plant Science Department which included Lesley Kellock, for her advice in computing and demonstrations of experimental techniques; Hugh Cairncross for his invaluable assistance in the propagation and maintenance of plant material; Robert Redpath and Alison Hendry for their technical assistance, and other members of the School of Agriculture, too numerous to mention, who have helped me in this work.

In addition, I would like to thank Frances Anderson for typing the manuscript.

Appreciation is extended to the Department of Agriculture and Fisheries for Scotland for their financial support during the period of this work, and to the many seed companies and the NVRS gene bank who donated seed samples.

Dedicated to my wife Jennifer,
my children Tracy and Tyler,
and my parents without whose patience,
understanding, support and love
this work would not have been possible

SUMMARY

In inoculation tests with *E. cruciferarum* only members of the families Cruciferae and Papaveraceae were infected: no infection occurred on plants selected from seven other families. Various cruciferous weed species showed a variety of susceptible to resistant responses in infection studies. From tests on a comprehensive range of cultivated cruciferous hosts with different isolates of *E. cruciferarum*, collected from different hosts and sources, differences in disease levels following inoculation were evident both between and within species. Levels of resistance or susceptibility of particular hosts tended to be reflected over the whole range of isolates, although some variation in the overall level of disease development associated with particular isolates occurred. Variation among isolates was generally quantitative rather than qualitative. Specific interactions with large effects occurred only occasionally within host species at a cultivar level. The development of different isolates on the susceptible cultivar Doon Major (*B. napus*) was similar and no relationship was found between the rate of colony development and the level of early production of conidia. The mean length of conidia of different isolates ranged from 42 to 45 µm and the mean breadth from 14 to 20 µm.

Rates of germ tube and appressorium formation were similar for all pathogen/cruciferous host combinations. Doon Major (*B. napus*) was recognised as a universally susceptible host and all other hosts tested exhibited some degree of resistance expressed at complete or partial levels. Complete resistance prevented growth beyond the appressorial stage although it was never found to occur with all infection units. Partial resistance emerged as causing a delay or restriction in

colony development and a reduction in spore production. Resistance may also be related to reduced haustorial production and efficiency. Various tissue responses to infection were observed. Early cell necrosis was associated with restricted infection, while in susceptible plants necrosis occurred at advanced stages of infection due to cell exhaustion. Callose deposition occurred with all hosts as a generalised response to penetration, but in some cases encapsulation of haustoria by callose was observed and associated with host resistance. Lignification occurred at sites of penetration and in lateral walls but no correlation between lignification and resistance was found. Factors which influenced leaf surface characteristics were found to affect the predisposition of host tissues to infection by *E. cruciferarum*.

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1. GENERAL INTRODUCTION

Powdery mildew disease of cruciferous crop plants, caused by *Erysiphe cruciferarum* Opiz ex. Junell, has been known in Britain for over 100 years (Smith, 1880) and yet has only recently been recognised to be of economic importance. The aim of this study is to develop further an understanding of the relationships between the biotrophic fungus *E. cruciferarum* and its hosts, with particular reference to the variation in host range within this pathogenic species, the fungal development characteristics found on different hosts and host tissue responses associated with susceptible or resistant forms in the family Cruciferae, the major host group.

The family Cruciferae contains some 220 genera and over 1900 species and, of these, a small number are of economic importance (Gill and Vear, 1980). Many of the cruciferous crops, of which *Brassica* is by far the most important genus, have been in cultivation for substantial periods of time (Prakash and Hinata, 1980): the Chinese book *Shih Ching*, said to have been edited by Confucius (551-479 BC), unmistakably mentions the turnip (Keng, 1974).

Cruciferous crops can be categorised into three types (Crisp, 1976):

(i) vegetables and salads for human consumption;

(ii) forage and fodder crops for animal feeds;

(iii) seed crops for oils and mustard condiments.

A feature of the Cruciferae is that many species contain crops in more than one group. *B. oleracea* is a European species which has been selected to give a wide range of crop types where leaves (kale), terminal buds (cabbage), axillary buds (Brussels sprout), swollen stems (kohlrabi) and floral tissues (cauliflower, broccoli) are harvested; *B. napus* occurs

in Europe as a vegetable or animal feed with swollen roots (swede), as a leafy fodder crop (rape kale) or as an oil seed crop (oilseed rape); *B. campestris* contains vegetable forms which have been selected in Asia for large terminal buds, swollen stems, swollen roots or large inflorescences; *B. juncea* includes forms with swollen stems or swollen roots. Both *B. campestris* and *B. juncea* have also been developed as leafy vegetables analogous to the kales of *B. oleracea* and as oil seed crops analogous to those of *B. napus* (Crisp, 1976).

Cytogenetical studies (Takamine, 1916; Karpechenko, 1922; U, 1935) have revealed that of the six agriculturally important *Brassica* species, three are essentially diploid and referred to as elementary species, viz. *campestris* (n=10), *nigra* (n=8) and *oleracea* (n=9). The three high chromosome species, *carinata* (n=17), *juncea* (n=18) and *napus* (n=19) were shown to be allotetraploids with their origins in interspecific hybridisations (Morinaga, 1933; 1934a,b) and consist of any two of the three elementary genomes. Morinaga assigned the genome symbols A, B and C to *campestris*, *nigra* and *oleracea* respectively, while the amphidiploids were designated AB (*juncea*), AC (*napus*) and BC (*carinata*). The cytogenetic relationship was later confirmed by U (1935) (Figure 1.1).

In most species sporophytic incompatibility systems prevent the satisfactory growth of pollen tubes following self-pollination, consequently cross-pollination is normally necessary to ensure fertilisation (Gill and Vear, 1980). However, *B. napus* cultivars are predominantly self-fertilised (Josefsson, 1948), although some outcrossing occurs under natural conditions (Palmer, 1962).

Cruciferous genera in addition to *Brassica* which include crop species are *Raphanus* and *Sinapsis*. *R. sativus* (n=9, genome R) is

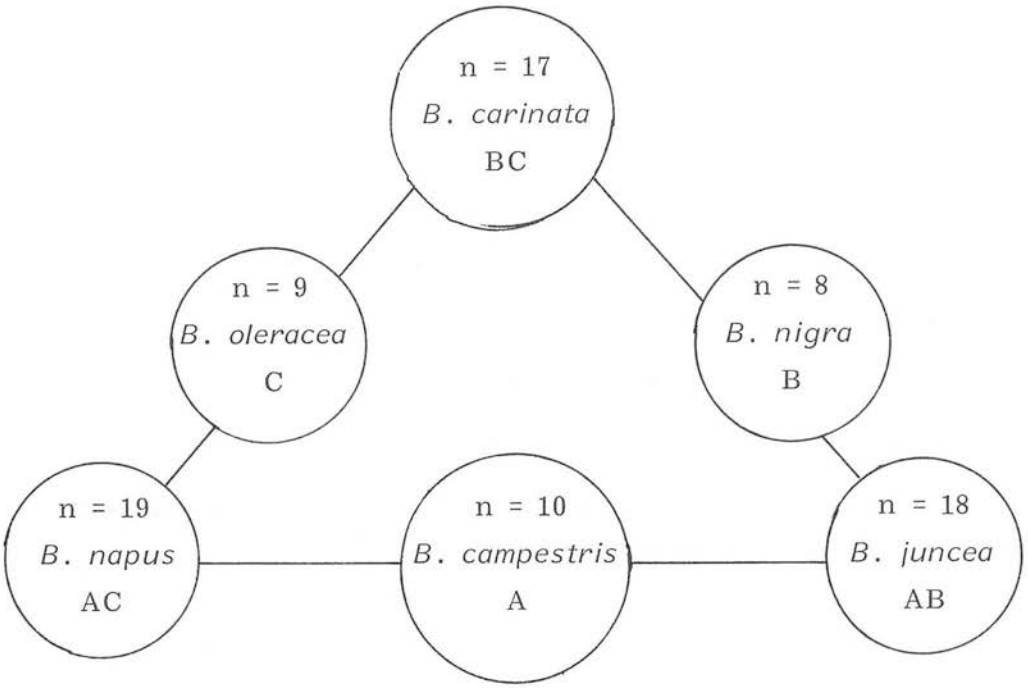


FIGURE 1.1: Cytogenetic relationships of *Brassica* species (from U, 1935).

closely related to the crop *Brassicas*, with the R genome partially homologous with the A and C genomes of *B. campestris* and *B. oleracea* respectively (Fukushima, 1929; Karpechenko, 1937). Crosses between *R. sativus* and *B. oleracea* have generated interest in the potential of the hybrid, *Raphanobrassica*, as a leafy forage crop (Gill and Vear, 1980).

The family Cruciferae also contains a number of agriculturally important weed species; Hanf (1972) lists 30 species as troublesome weeds, amongst which some of the more common include *Sinapsis arvensis* (charlock), *Capsella bursa-pastoris* (shepherd's purse), *Sisymbrium officinale* (hedge mustard), *Arabidopsis thaliana* (thale cress) and *Thlaspi arvense* (common penny-cress).

E. cruciferarum can infect all photosynthetic tissues of cruciferous crops, for example swollen roots, stems, leaves, axillary buds, terminal buds and seed capsules. Infection of both upper and lower leaf surfaces

can occur, but is more commonly found on the upper surface. Powdery mildew appears first as small star-shaped lesions on the leaf surface. The mycelium, which remains superficial, ramifies over the leaf surface from isolated colonies gradually coalescing until the entire leaf surface is covered, giving the leaf an off-white, floury appearance and providing a source of spores for further air-borne spread of the disease.

Under favourable conditions, following conidial deposition onto a host leaf, germination of *E. cruciferarum* conidia may occur within 2 hours to form a short germ tube and by 8 hours this may develop an appressorium, usually formed close to the point of spore alightment (Brain, 1978). A single germ tube develops from one corner of a conidium of *E. cruciferarum* and terminates in a moderately lobed appressorium (Boesewinkel, 1979) (Plate 1.1). After the appressorium becomes firmly attached to the leaf surface, penetration occurs directly through the cuticle by downward growth of a thin hyphal thread (Plate 1.2) from the lower surface of the appressorium (Tarr, 1972). Once the infection hypha has penetrated the cell wall, a globular haustorium is formed within the host cell (Plate 1.2). Mature haustoria of *E. polygoni* were detected in epidermal cells of clover leaves 9 hours after inoculation by Smith (1938).

With *E. cruciferarum* conidia a primary hypha is produced after 16 hours, at the same end as the appressorium, and after 24 hours a second hypha is formed at the distal end (Brain, 1978). By 48 hours further hyphae are present and the mycelium branching and extending. Sporulation begins within 2 to 4 days (Brain, 1978).

Powdery mildew disease of *Brassica* crops does not generally appear until late summer. However, in areas which tend to be warmer and drier the disease can be found earlier. The onset of infection and

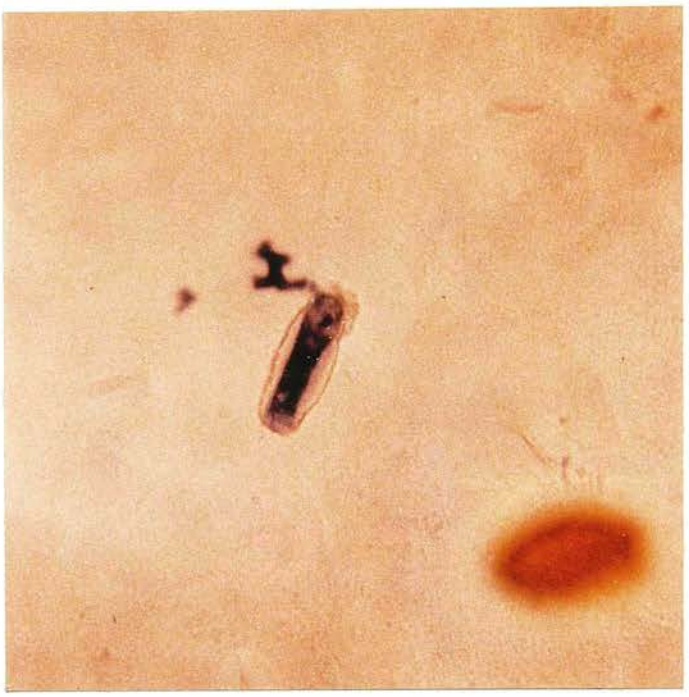


PLATE 1.1: *E. cruciferarum* conidium with lobed appressorium (x 62.5)*.

PLATE 1.2: Penetration hypha and globular haustorium of *E. cruciferarum* (x 250).

*In the plates the magnifications given refer to the microscope lens and not to actual size.

severity of the disease is very much affected by prevailing weather conditions. In cool, wet seasons the disease is of little importance (Anon, 1978), while Moore (1959) showed there to be a correlation between hot, dry summers and severe outbreaks of the disease. The influence of weather on the disease is such that a change from hot, dry to cool, wet conditions may lead to a natural recession of the pathogen and recovery of the crop (Rodger and Purdie, 1977).

Experiments by Purnell (1971) on the effect of temperature on powdery mildew development showed there to be very little growth of *E. cruciferarum* at temperatures below 10°C, that vegetative growth was good only at temperatures above 15°C, and little sporulation occurred below 18°C. The most rapid vegetative growth and sporulation occurred between 18° and 22°C.

The potential yield loss attributable to mildew infection has been quantified by several workers: the application of fungicides to swedes has given increases in yield (dry matter/ha) of between 20 to 33% (Anon, 1976a,b; French, Nichols, Wilson, Rogerson and Wright, 1977). Moreover, losses are due not only to an immediate reduction in yield but also to a loss of quality and consequent downgrading. Accurate information on the financial loss to growers through reduced quality are difficult to obtain. However, Dixon (1978) considered that heavy losses occurred as indicated by the readiness of growers to use fungicidal controls. For many years, sowing swedes late to avoid the worst epidemics of *E. cruciferarum* was practised. However, several workers (Anon, 1969; Dixon and Furber, 1971; Munro and Scourey, 1976) demonstrated that by delaying sowing, and thus shortening the growing season, a considerable loss in yield results. Although delayed sowing is moderately successful in avoiding infection (Dixon, 1981), the penalty

from reduced yields is great. Experiments have shown that each week's delay in sowing results in losses from 4.3 to 5.9 t/ha (Anon, 1969; Munro and Scourey, 1976). In addition, as severe outbreaks of powdery mildew occur only once every few years (Dixon and Furber, 1971), the potential yield loss is even higher.

Fungicidal control of powdery mildew on *Brassica* crops is a widely accepted practice. With swedes, treatment when mildew leaf infection is 5% can give yield increases of nearly 23%. Furthermore, a second fungicide application may increase yield by a further 6-10% (Anon, 1976a,b; French *et al.*, 1977).

Following a description of general materials and methods the experimental studies are described in four sections. The first aspect considered is the range of variation in *E. cruciferarum*. This is followed by an account of fungal development patterns and host tissue responses which may be associated with resistance/susceptibility. In the subsequent section, the responses of host cells to infection is examined further in histochemical studies. Various sources of possible variation in host responses to infection within cultivars is considered in the final experimental study.

2. GENERAL MATERIALS AND METHODS

2.a Isolate preparation and maintenance

The isolates of *E. cruciferarum* used in this study were collected from different host plants and from different locations over a wide area (Table 2.1). After collection each isolate was bulk inoculated (Smith, 1938) onto leaf discs of the swede cultivar Doon Major, which proved to be universally susceptible to *E. cruciferarum*. Individual isolates were grown on leaf discs (1.8 cm diameter) placed with the upper surface uppermost in separate 10 cm square petri dishes containing 0.36% Davis agar, incorporating 100 ppm benzimidazole in order to delay leaf senescence (Person, Samborski and Forsyth, 1957).

After transferring the original collections to Doon Major leaf discs, the isolates were incubated at 18°C (Purnell, 1971) under a 16 hour light (81 Lux) : 8 hour dark regime until sporulation occurred. Twenty-five single spores of each isolate were then removed from the sporulating colonies, using an eyelash embedded into a wax-filled pipette whilst viewing through a stereoscopic zoom binocular microscope. Each single spore was inoculated onto a separate leaf disc. After incubating for 5 days, one sporulating colony/isolate was chosen at random and a further 10 single spore inoculations carried out. Again, after incubation, a single sporulating colony/isolate was chosen at random and spores from this were bulk inoculated onto Doon Major leaf discs. Once cultures had been established, each isolate was maintained on leaf discs in individual 3.5 cm diameter petri dishes on benzimidazole agar. Every 2 to 3 weeks all isolates were sub-cultured onto healthy Doon Major leaf discs, and every 8 weeks each isolate was cultured for a stage on its original host.

To avoid the risk of contamination all plants used for inoculation were raised in domed pots on an isolation propagator (Jenkyn, Hirst

TABLE 2.1: Index of isolates of *E. cruciferarum* and their sources.

Isolate reference index	Original host			Nearest town	Region
	Species	Crop	Cultivar		
N1	*Brassica napus*	Swede	Conqueror	Penicuik	Lothian
N2a	"	"	Marian	"	"
N2b	"	"	Askgarde	"	"
N2c	"	"	Doon Major	"	"
N2d	"	"	Acme	"	"
N2e	"	"	Magres	"	"
N2f	"	"	Criffel	"	"
N3a	"	"	Doon Major	Aberdeen	Grampian
N3b	"	"	Merrick	"	"
N4a	"	Rape kale	Rape Kale	Edinburgh	Lothian
N4b	"	Swede	Western Perfection	"	"
N4c	"	"	Mancunian	"	"
N5	"	"	Unknown	Cambridge	Cambridgeshire
N6	"	"	9943	Inverness	Highland
O4a	*B. oleracea*	Brussels sprout	Fasolt	Edinburgh	Lothian
O4b	"	Kale	Pentland Brig	"	"
O7a	"	Brussels sprout	Lunet	Duns	Border
O7b	"	"	Citadel	"	"
O8	"	"	January King	Dalkeith	Lothian
C5	*B. campestris*	Turnip	Wallace	Cambridge	Cambridgeshire
W9	*Sinapsis arvensis*	Charlock	-	Edinburgh	Lothian

and King, 1973) and all leaf discs were cut and inoculations carried out in a laminar flow cabinet.

Spores used for inoculation in the experimental studies were produced in either of two ways.

(i) Method A

Each isolate was bulk inoculated onto 25 Doon Major leaf discs on benzimidazole agar 14 days before the inoculum was required. The 25 discs/isolate were incubated in individual 10 cm square petri dishes as previously described. To ensure that only fresh viable conidia were available, plates were shaken in order to dislodge any old conidia 24 hours before the inoculum was to be used.

(ii) Method B

Isolates were grown on Doon Major plants in domed pots of the isolation propagator. Half the number of infected plants were replaced every 12 weeks with healthy 4 week old Doon Major plants which were inoculated by dusting with spores of previously infected plants. The propagator was maintained at a temperature of 20°C ± 5°C, under natural light conditions.

Inoculum used in any one experiment was obtained entirely by either method A or method B.

2.b Inoculation techniques

Two inoculating techniques were employed in the experimental work. For studies on the host range of isolates a brush inoculation technique was applied while, for all other experiments, spore settling towers were used.

(i) Brush inoculation

In the host range studies, where up to 21 isolates were being inoculated onto leaf discs within a short space of time, transferring spores with a sterile brush was found to be the most convenient and appropriate method. Inoculations were carried out within a laminar flow cabinet. To prevent spores being carried away from the leaf surface by the strong air current within the cabinet, the leaf discs to be inoculated were surrounded by a glass frame 8 cm high. Infected leaf discs were held within the frame 1 - 1.5 cm above the surface of the uninfected leaf discs and spores were transferred by a small sable hair brush, previously sterilised by soaking in absolute alcohol and allowed to dry within a spore free laminar flow cabinet. The cabinet was run for a period of 4 minutes between each isolate inoculation to clear the cabinet of any spores. The glass frame and cabinet base were sprayed with absolute alcohol after each time of use.

(ii) Spore settling tower techniques

(a) In several studies a technique based on that of Brain (1978), using a large spore settling tower, was employed. The tower had an overall height of 168 cm and consisted of two aluminium cylinders, 56 cm in diameter, separated by a spore trap mechanism in the form of a sliding aluminium shutter. The tower was covered with a tight fitting aluminium lid over the top cylinder and stood on a draught-free, flat aluminium base. A length of copper tubing, 1 cm in diameter, bent at a right angle to give a vertical 7 cm stem positioned centrally in the tower was introduced from the side of the upper cylinder 75 cm from the cylinder base (Figure 2.1). Plant material for inoculation was positioned on the tower base and the spore trap closed. Spores from

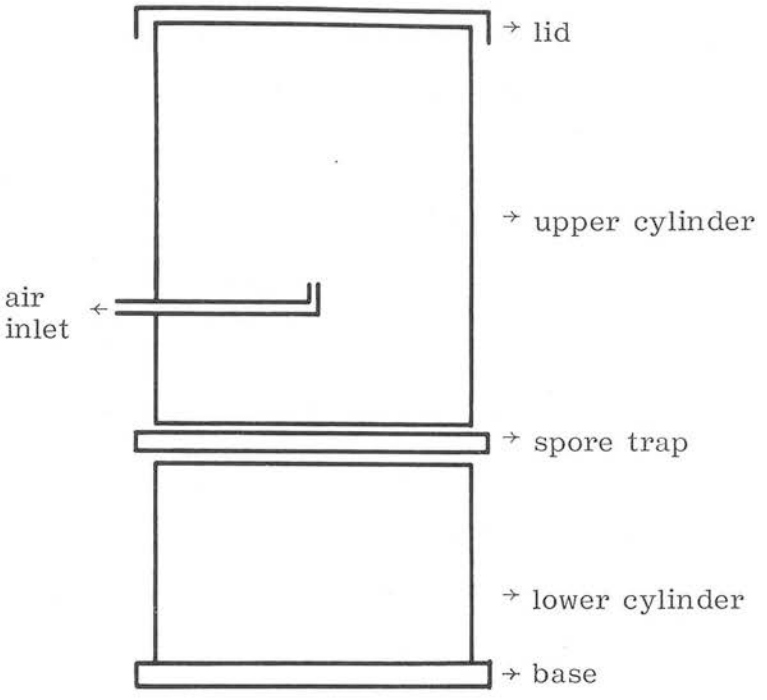


FIGURE 2.1: Diagram of spore tower apparatus.

a heavily infected plant were shaken into the top of the tower and, after replacing the lid, a blast of air was passed along the copper tube to force the inoculum into a spore cloud. After 30 seconds the spore trap was opened and deposition allowed onto the test plant material for a period of 5 minutes. The initial 30 second delay was to ensure that any debris or large spore clumps would be caught in the trap. Inoculum for use in this settling tower was produced by method B.

(b) When smaller amounts of test material were required to be inoculated a small settling tower was used: this consisted of a single aluminium cylinder 76 cm high and 23 cm in diameter. A glass tube, bent at right angles to give a vertical 2.5 cm stem inside the tower in a central position, was introduced 10 cm from the tower base. Material for inoculation was enclosed on the base of the tower and spores were introduced at the top from either infected leaves or leaf discs. Following the introduction

of spores the tower was closed by a lid and a blast of air forced through the glass tube. Spore deposition was allowed onto the test material for 2 minutes.

2.c Host test material

(i) Growth and development conditions

Plants of test cultivars were raised in Levington's potting compost in 30 cm seed trays. Fourteen days after sowing, seedlings were transplanted into 13 cm plastic pots. From these, representative plants of uniform size were chosen for experimentation. Test plants were grown in a glasshouse where the winter period (October - April) temperature ranged between 10-20°C with a mean of 15°C, whereas during the summer period (May - September) the temperature ranged between 10-30°C with a mean of 20°C. During the winter period natural light was supplemented by four mercury vapour lights (4325 Lux) for 12 hours. No supplementary light was given during the summer period. Plants were watered as required. To control aphids, test plants were sprayed with either Hostaquick (55% ec heptenophos, 1 ml/l l water) or Pirimor (50% w/w pirimicarb, 0.5 g/l l water). In experiments where the test material was to be inoculated 7 weeks after sowing, a single application of Hostaquick was applied. In all other experiments plants received two applications of Hostaquick, with the exception of experiments on plant maturity (Section 6) and seed source (Section 6), in which two applications of Pirimor were given.

In experiments where test plants were to be grown for periods longer than 7 weeks after sowing, a liquid fertiliser was given once every week from the sixth week after sowing. Sangral (10 ml/5 l water) liquid fertiliser was used in all experiments except the first study on

plant maturity (Experiment 6b) which received Solufeed (base solution 680 g/4.5 l water, further diluted with water 1 : 100). Both liquid fertilisers were utilised in Experiment 6e.

(ii) Choice of test material

Brain (1978) used the Leaf Plastochron Index method (Erickson and Michelini, 1957) in the selection of leaves for infection studies, on the basis of developmental status rather than of chronological age. Brain was able to implement this method as he was experimenting with only the one crop, swede. In this study cultivars from different crops, sub-species and genera were to be examined, and the Leaf Plastochron Index method was inappropriate. The growth habit of plants belonging to the family Cruciferae, of alternate leaf development, enables leaves of different chronological age to be readily identified. Throughout this experimental study, unless otherwise stated, the oldest true green leaf was chosen as the test material from each cultivar grown. Intra-cultivar variation associated with the predominance of outbreeding among members of Cruciferae (Simmonds, 1976) was recognised to provide a source of uncontrolled experimental variation. By using leaf discs, even when many isolates were to be tested on each cultivar as in the host range studies, all discs of a single replicate could be cut from a single test leaf to reduce this source of error. Moreover, by using leaf discs the smaller volume of space involved made it easier to provide uniform environmental conditions in tests. In taking discs, the midrib and major veins were avoided and the risk of contamination was reduced by sampling leaves in a laminar flow cabinet.

Leaf discs were removed using a 1.8 cm diameter cork-borer and placed with the upper leaf surface upwards on benzimidazole agar.

In all experiments leaf discs were incubated in 10 cm square petri dishes within which their position was assigned randomly. Similarly, all plates of different experimental treatments within each replicate were randomly positioned in a controlled environment growth cabinet. Temperature conditions of 18°C ± 2°C with a 16 hour light (81 Lux) : 8 hour dark regime were maintained within the cabinets in all experiments.

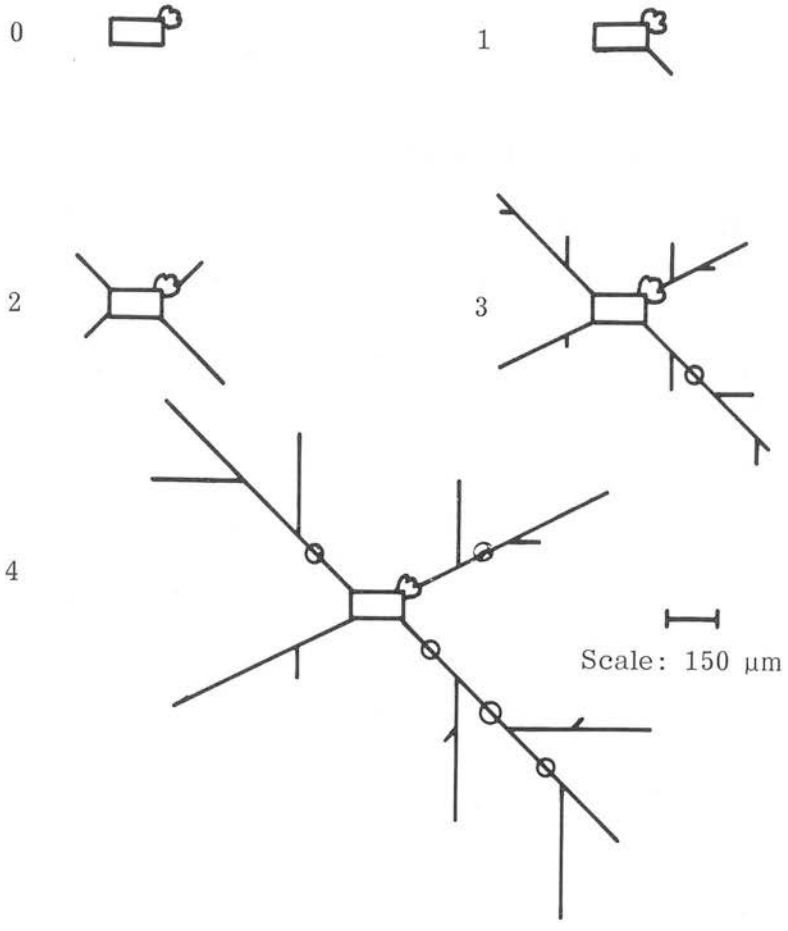
2.d Disease assessment and fungal development

(i) Disease assessment

In 1971, Purnell used a 0-4 Disease assessment scale to microscopically assess mildew growth 18 days after inoculation. In this study, this method was modified and a 0-5 Disease assessment scale (Figure 2.2) was devised to describe the level of mildew growth 6 days after inoculation, using a stereoscopic zoom microscope. In characterising the infection type of any particular host/isolate combination, the extent of disease at 6 days attained by the majority of spores examined was taken. In all cases this majority was found to be at least 75% of the total infection units observed. A minimum number of 50 spores was examined.

(ii) Fungal development

In some experiments fungal development from germination through to sporulation was studied microscopically. In preparing a recording scheme, some diversity was encountered in the literature concerning the terminology used to describe the early growth stages of a developing mildew colony. After appressorium formation, there are usually three to four hyphae produced from each conidium, depending on the species. These elongate and branch to form the mycelium of the fungus. Delp



0 Spore germination, no fungal growth beyond appressorium formation.

1 Restricted mycelial growth, no sporulation.

2 Limited mycelial growth, no sporulation.

3 Moderate mycelium, limited sporulation.

4 Abundant mycelium, moderate sporulation.

5 Abundant mycelium, abundant sporulation.

FIGURE 2.2: Diagrammatic representation of the 0-5 Disease assessment scale.

(1954), using *Uncinula necator*, favoured the terms primary, secondary and tertiary, for those hyphae formed from the conidium subsequent to initial infection. Smith (1969), in cross-inoculation experiments with *Erysiphe polygoni*, did not use the term hyphae but described, after primary germ tube formation, the production of up to four secondary germ tubes which form the mycelium as they begin to branch. A further descriptive term was introduced by Mount and Ellingboe (1966) who used the expression secondary hyphal initials for any hyphae produced after appressorium formation: as these elongate they form functional secondary hyphae. According to Manners (1966), a structure might be considered a germ tube for as long as it obtains nutrients from the spore, after which time it becomes a hypha. This may be a sound definition, but it is difficult to determine when the supply of nutrients in the germinating spore is exhausted.

Purnell (1971) adopted the terms designated by Delp (1954) for reference to hyphae from a germinated conidium in a description of the pattern of development of *E. cruciferarum* (Figure 2.3). Brain (1978), on the other hand, used a scoring system for *E. cruciferarum* development which was based on the stage of fungal growth reached for each conidium as referred to by Manners and Hossain (1963), Manners (1966) and Purnell (1971). This may be summarised as follows:

Symbol	Fungal growth stage
-	Ungerminated
G	Germinated (germ tube length > breadth)
A	Appressorium formed
P	Primary hypha present
S	Secondary hypha present
+	Expanding young colony (largest diameter 0.4 mm)

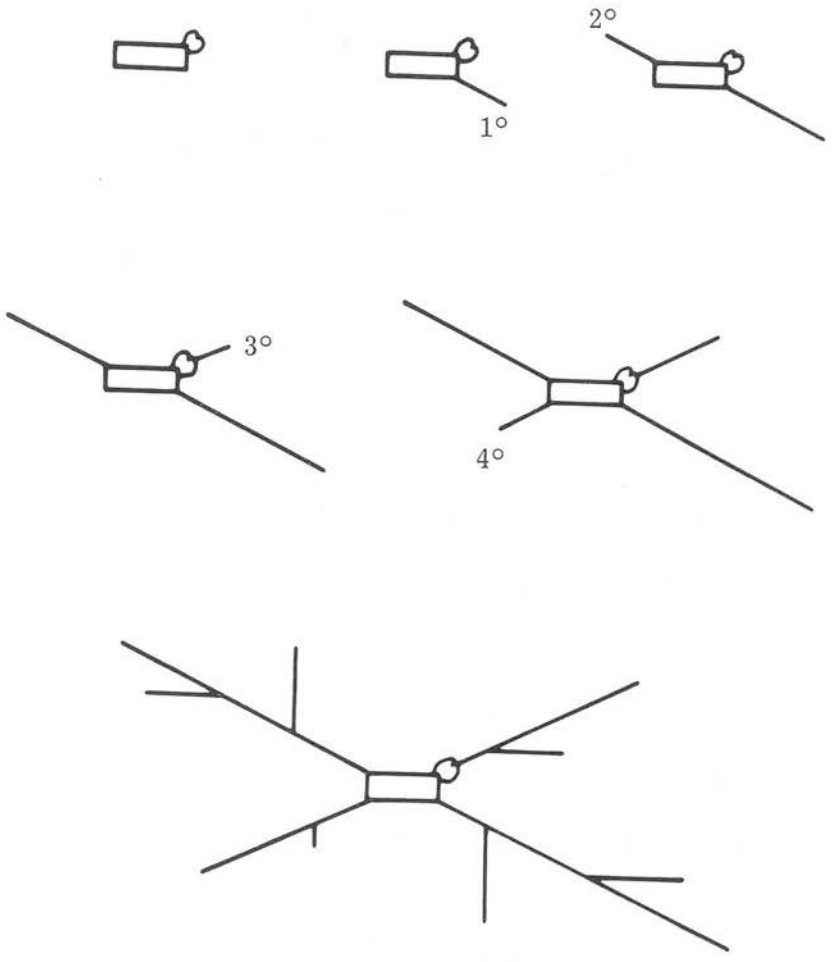


FIGURE 2.3: Pattern of hyphal development adopted by Purnell (1971).

In this study, the system of Brain (1978) was extended to include the categories conidiophore initials and conidia as follows:

Symbol	Fungal growth stage
UG	Ungerminated
G	Germ tube
A	Appressorium formed
PH	Primary hypha present
SH	Secondary hypha present
EC	Expanding young colony
Ci	Conidiophore initials present
Co	Conidia present

The definition of primary hypha and secondary hypha differed from that adopted by Purnell (1971). This was felt necessary to enable the progress of colony development to be studied in more detail and, as a result, a more comprehensive method for hyphal identification was formulated.

The four hyphae which Purnell (1971) designated as primary, secondary, tertiary and quarternary were, in this study, defined as primary A (1°A), primary B (1°B), primary C (1°C) and primary D (1°D). This nomenclature enabled further hyphal development to be categorised. Thus, all hyphae which originated from either of the four primary hyphae were identified as secondary hyphae (2°), and assigned a letter (A, B, C or D), thereby indicating from which primary hypha they originated. The same procedure was applied to all tertiary hyphae (3°) which developed from secondary hyphae.

A hyphal development pattern not described by Purnell (1971) was one in which no 1°D hyphae developed. However, in these cases, a third hypha developed from the appressorial end of the germinated conidium. This hypha was described as primary X (1°X) and the nomenclature for 2° and 3° hyphae developing from the 1°X hypha followed the approach described above (Figure 2.4).

2.e Statistical analyses

In biological experiments there are many sources of variation which can be anticipated but not eliminated. Some variation may be reduced, such as in the present studies by using discs from the same leaf of a cultivar for different inoculation treatments or by using controlled environment incubators. Randomised block designs usually with four replicates were used in all experiments and an analysis of

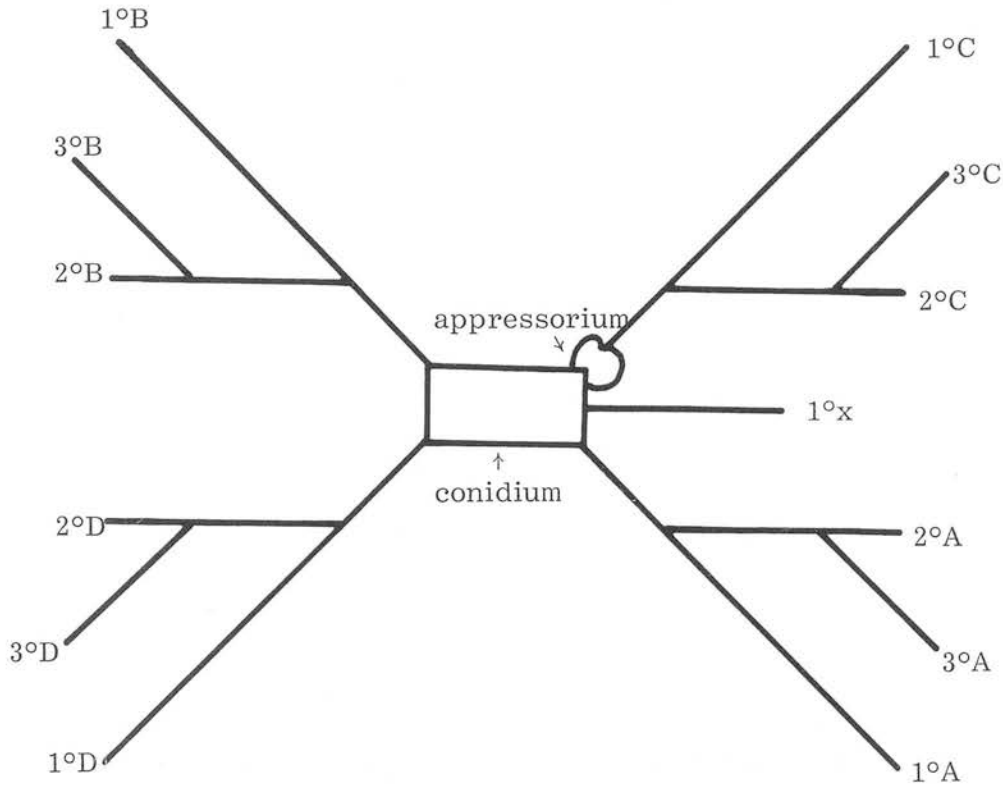


FIGURE 2.4: Nomenclature for 1°, 2° and 3° hyphal development, in this study.

variance was applied to the data from each experiment using the Genstat Statistical Programme (Anon, 1980).

3. VARIATION IN *ERYSIPHE CRUCIFERARUM*

INTRODUCTION

The first naming of a powdery mildew as a specific organism was probably that attributed to Linnaeus (1753), when he gave the binomial *Mucor erysiphe* to a white fungus on the leaves of *Humulus*, *Acer*, *Lamia*, *Galeopsis* and *Lithospermum*. The name *Mucor* was soon dropped as the generic epithet of powdery mildew fungi, since it had previously been applied to a clearly distinct fungus. *Erysiphe* was introduced as a generic name and later as a family name (Erysiphaceae) applied to the group.

De Candolle (1805, 1815) was the first to describe mildew species extensively: he named 25 new species of *Erysiphe*. A little later Wallroth (1819a,b) included all 25 in the genus *Alphitomorpha*. However, many of the species described by De Candolle (1805, 1815) are still in use today.

In 1829, Schlectendahl distinguished species with one ascus from species with several; later Castagne (1845) observed that the numbers of ascospores was not the same in all species. Their observations culminated in the work of Leveille (1851) who grouped the species into six genera (*Calocladia*, *Erysiphe*, *Phyllactinia*, *Podosphaera*, *Sphaerotheca* and *Uncinula*) according to their asci, ascospores and cleistothecial appendages, thereby setting up the first taxonomic system with a key for the genera of powdery mildews.

Almost 50 years later Salmon (1900) published a monograph of the Erysiphaceae of the world which, except for changing *Calocladia* to *Microsphaera*, is identical to the classification by Leveille (1851). Salmon's key to the genera and species of powdery mildew are probably the most widely used today (Yarwood, 1978).

E. communis in the sense of Fries (1829) is a comprehensive species, including almost the whole genus *Erysiphe* in its present circumscription, thus mainly agreeing with Wallroth's (1819a) *Alphitomorpha communis* (Junell, 1967). Confusion associated with *E. communis* has, in part, been caused by the species being split by several authors (Leveille, 1851; Salmon, 1900; Blumer, 1933; Junell, 1967) resulting in a large number of new species, only a few of which are widely accepted, the remainder succeeding only to cloud the nomenclature. Salmon (1900) considered *E. communis* to be a synonym of *E. polygoni*. The latter species is, however, accepted in a more restricted sense by Blumer (1933) and is excluded from *E. communis*.

The names *E. communis* and *E. polygoni* are both in use today: some taxonomists consider them to be distinct, whereas others consider them to be synonomous. Moreover, both species have been split into further species and varieties. The identity of divisions within and between *E. communis* and *E. polygoni* remains uncertain due to the inconsistent results obtained by different workers who used dissimilar morphological criteria, inoculating techniques, incubation conditions, isolates of the pathogen, and sources of host material. Consequently, the taxonomy and nomenclature of *E. communis* and *E. polygoni* remains inconclusive.

Powdery mildew fungi are all obligate parasites but the degree of host specificity varies with different species. Within this group of fungi host specificity is expressed at different levels: some species have apparently a wide host range, infecting plant species from several different families (e.g. *E. cichoracearum*, *E. polygoni*, *S. fuliginea*), while others are found to be specific at the family level (e.g. *E. graminis*, *E. horridula*, *S. macularis*). Moreover, there are mildew species which

infect only members of a single genera, such as *E. betae* infecting *Beta* species and *E. circeae* infecting *Circaea* species (Junell, 1967).

Pathogenicity denotes the ability of a parasite to injure a host (Scott, Johnson, Lowe and Bennet, 1978): using host specific pathogenicity as a basis to set apart isolates of a single species, Marchal (1902) distinguished, on their ability to infect species from a single genus, seven *formae speciales* within *E. graminis*. Within the *formae speciales* of *E. graminis* the host range is often not as narrow as the *forma specialis* epithet implies (Jenkyn and Bainbridge, 1978). For example, Hardison (1944) found that all the cultures of *E. graminis* which he studied produced infection on species in two or more genera. More recently, Eshed and Wahl (1970) found that *formae speciales* of the cereal mildews possessed a relatively wide host range.

Specialisation of parasitism within *formae speciales* of *E. graminis* was first reported in 1930 (Mains and Deitz; Waterhouse). This variation in cultivar specific pathogenicity between isolates within a *forma specialis* is identified on the basis of their interactions with a selection of different host cultivars, *viz.* a differential host series. Isolates which differ in their interactions with such a differential series are described as belonging to different physiologic races, i.e. races which are morphologically indistinguishable but differ in their physiology (Scott *et al.*, 1978). Yarwood (1957) considered that physiologic race specialisation was to be expected in all morphological species of powdery mildews, and predicted that it would be present in species not studied. For example, even though the species *E. cichoracearum*, *E. polygoni* and *S. fuliginea* appear to have little host specificity, there are reports of the presence within each species of isolates which have a much narrower host range and, also, that the host range varies between

different isolates (Yarwood, 1957; Stone, 1962; Stavely and Hanson, 1966).

It was only relatively recently that *E. cruciferarum* was delimited by Junell (1967) as being distinct from *E. communis*, an aggregate species with an extremely diverse and wide host range. Junell distinguished *E. cruciferarum* on the basis of sexual and asexual characteristics and host range. She listed as hosts of *E. cruciferarum*, 60 species from 33 genera of the family Cruciferae and six species from the family Papaveraceae. Additional hosts have been reported by Boesewinkel (1979) from the families Cleomaceae, Fumariaceae, Geraniaceae and, more recently, four species of *Rhododendron* together with species of *Erica*, *Aquaria*, *Cavendishia* and *Villardia* have also been listed (Boesewinkel, 1981). With such a diverse host range, it would appear that *E. cruciferarum* displays less host specificity than most powdery mildews. However, there are several reports of different strains of *E. cruciferarum* (Sawamura, 1957; Chupp and Sherf, 1960). Dixon (1977) observed that the pathogenic adaptation shown by *E. cruciferarum*, which enabled the resistance of the Brussels sprout cultivar Rampart to be overcome, may be indicative of the presence of physiologic races. Later he indicated that there may be sub-specific variants within *E. cruciferarum*, since it is difficult to transmit isolates of the fungus from Brussels sprout to turnip, and *vice versa* (Dixon, 1981).

When Junell (1967) erected the species *E. cruciferarum* she described the range of dimensions of conidia: several workers have since examined *E. cruciferarum* conidia and reported a much wider range of conidial size. Although conidial size has been considered in several investigations, relatively little work has been carried out on levels of conidial production, nor on patterns of colony development, of different isolates of *E. cruciferarum*.

The main objectives of the experiments within this section were to study the host range of a collection of *E. cruciferarum* isolates from different sources and to establish any evidence of variation in host specificity, to describe the pattern of colony development of different isolates on a compatible host and to assess, for some isolates, their levels of conidial production. Conidial size of different isolates was also considered. These various aspects are considered under the following headings:

3.a Host range of isolates

3.b Development patterns of isolates

3.c Conidial production of isolates

3.d Conidial size of isolates

MATERIALS AND METHODS

Experiment 3.a

To determine the host range of *E. cruciferarum*, isolates from different sources with respect to host and location were tested on a wide range of plants. Hosts were selected within the family Cruciferae, representing different genera, species, sub-species and cultivars (Table 3.1). A selection of species from different families (Table 3.1e) was also tested as potential hosts.

To facilitate such a large study the hosts to be tested were divided into groups, enabling all isolates to be examined simultaneously on each group of host plants. Initially it was planned to include only one crop type in each group and Group 1 contained nine yellow turnip cultivars. However, all subsequent groups tested were composed of several species as difficulty was found in ensuring synchronous development of inoculum of all isolates in any one test: this resulted in a risk of failure to test some isolates on one crop type and making it necessary to repeat the group. By testing several crop types in each group, isolates had more than one opportunity to be tested on any particular crop. The universally susceptible swede cultivar, Doon Major, was used as a control in each group of hosts tested, except Group VII.

All host test material was prepared and inoculated 7 weeks after sowing, except that of *Geranium zonale* where test material was obtained from established plants. Inoculum was prepared by method A, and leaf discs inoculated using the brush inoculation technique (Section 2.b). Following incubation for 6 days, each group of hosts was then assessed using the 0-5 Disease assessment scale. Four replicates of each host/isolate combination were used in each group test. Eight

group tests were carried out. Before the final test, when non-cruciferous plants were tested, the cooling system of the incubator used for isolate maintenance broke down, resulting in all isolates being destroyed. Consequently, the plants were tested with only one newly collected isolate. The procedure described to obtain a single spore culture was carried out with the newly collected isolate, prior to inoculation of the non-hosts.

Experiment 3.b

Ten isolates (Figure 3.4) were chosen from the collection used in the host range studies and inoculated by brushing conidia from leaf discs onto leaf discs of the cultivar Doon Major, which were then incubated under standard conditions for varying periods of time. Discs of each isolate were fixed and cleared in 95% ethanol at 4, 8, 12, 24, 48, 72, 96 and 168 hours after inoculation. The discs were mounted on slides in 0.1% trypan blue in lactophenol and examined microscopically. Using the Fungal Development Scale, assessment was made of the stage of development of 100 conidia on each of four replicate discs for each isolate at every time stage. Clumps of spores were ignored, only isolated spores being assessed.

Experiment 3.c

Doon Major plants and inoculum were produced as in Experiment 3a and inoculated in the small settling tower. Four isolates (Table 3.3) were selected for quantitative assessment of the production of conidia. An estimate was made of the level of inoculum on leaf discs inoculated by each isolate. This was achieved by counting the number of conidia in each of 20 fields of view on five leaf discs inoculated with a single

isolate. Knowing the total leaf disc area (255 mm^2) and the area of the field of view (3.14 mm^2), the mean number of conidia per leaf disc after inoculation by each isolate was calculated. The percentage germination of conidia of each isolate was assessed 24 hours after inoculation.

The number of conidia produced by each isolate on five leaf discs was counted at 4 days after inoculation and then daily from 6 days to 14 days. Counts of conidia production were obtained by placing each disc into a centrifuge tube together with 0.2 cm^3 distilled water (+0.5% Tween). Each tube was shaken on a Griffen test-tube shaker for 60 seconds and, with the aid of a haemocytometer, the number of conidia present in 10 counts/disc estimated.

Experiment 3.d

Fifteen isolates (Figure 3.10) from the collection in the host range experiments were inoculated onto leaf discs of the cultivar Doon Major 7 weeks after sowing, using the brush method. After incubation for 14 days in standard conditions, conidia present on sporulating colonies on the leaf discs were removed using a Humbrol air spray. Leaf discs were then incubated for a further 24 hours and newly produced conidia from four replicate discs of each isolate were brushed into drops of lactophenol on glass slides, using one slide for each replicate. The length and breadth of 200 conidia (50/replicate) of each isolate were measured under the microscope, using an eyepiece micrometer, and the length : breadth ratio calculated.

RESULTS

Experiment 3.a: *Host range of isolates*

Prior to analysis, the data of each host group assessment were transformed, using both $\sqrt{x+0.5}$ and the angular (x) transformations. However, neither transformation significantly affected the analyses and only the analyses of untransformed data are considered in the text. From the analysis of variance of the results of disease assessments for each group of cruciferous hosts tested, significant differences were found between hosts (Table 3.1) and, except for Group VI (cultivars of *B. campestris* and *C. draba* and *R. raphanistrum*), between isolates (Table 3.2). The interaction between these two factors was also significant in Groups II (cultivars of *B. oleracea* and *B. napus*), IV (cultivars of *B. napus*, *Raphanobrassica*, *R. sativus*, *B. juncea*, *B. carinata* and *S. alba*), V (cultivars of *B. oleracea* and *B. campestris*) and VII (mainly weed species), as indicated in Figure 3.1.

Table 3.1 summarises the average response of the different hosts to the various isolates tested: the hosts are arranged in their taxonomic categories while the test group in which they were assessed is indicated along with the mean for Doon Major, as a highly susceptible control in each particular group assessment concerned. It may be noted that the disease indices for Doon Major varied in the different tests and thus the results for hosts assessed at different times are not directly comparable. The tables also indicate isolates which gave infection levels substantially above or below average for a particular host. Differences in infection levels were evident between host varieties or cultivars of *B. campestris* (Table 3.1a), *B. napus* (Table 3.1b) and *B. oleracea* (Table 3.1c). Other cultivated species tended to show little or no

TABLE 3.1: Disease indices for different plant species and cultivars inoculated with different isolates of *E. cruciferarum*.

Host	Disease Index mean (± SED)*	Doon Major Disease Index	Group test number	Isolates with indices substantially different from average above	below
(a) *B. campestris* cultivars					
var. *pekinensis* (Chinese cabbage)					
50 Days	1.5 *(0.46)*	3.7	VI		
Spring A1	0.0 *(0.46)*	3.7	VI		
Tip Top	2.3 *(0.46)*	3.7	VI		
var. *rapifera* (stubble turnip)					
Civasto	1.5 *(0.46)*	3.7	VI	N2e	
Debra	1.9 *(0.46)*	3.7	VI		
Ponda	2.2 *(0.54)*	3.9	V	N6	
Tyfon	1.6 *(0.54)*	3.9	V	N6	
var. rapifera (yellow turnip)					
Aberdeen GTY	0.5 *(0.68)*	3.8	I	N2c	
Balmoral	1.2 *(0.68)*	3.8	I	N2c	
Brimmond	1.3 *(0.68)*	3.8	I	N2e	
Bruce	0.5 *(0.68)*	3.8	I	N2e,N2c	
Findlay	0.8 *(0.68)*	3.8	I	N2e,N2c	
Green Top Scotch	0.3 *(0.68)*	3.8	I	N2c	
Invincible GTY	1.2 *(0.68)*	3.8	I		
Manchester Market	0.2 *(0.68)*	3.8	I	N2c	
Wallace	1.4 *(0.68)*	3.8	I		
*DF: Group I = 477; Group V = 510; Group VI = 357					
(b) *B. napus* cultivars					
var. *oleifera* (forage rape)					
Barsica	0.7 *(0.54)*	2.9	II	N2f	
Maris Haplona	2.7 *(0.64)*	3.0	III		
ZL22	1.6 *(0.54)*	2.9	II		
var. *rapifera* (oilseed rape)					
Brutor	1.6 *(0.54)*	2.9	II	N2c	
Loras	1.6 *(0.54)*	1.8	IV		
Willi	0.7 *(0.54)*	2.9	II	N2e	
var. *rapifera* (swede)					
Marian	1.5 *(0.54)*	1.8	IV		O4a
Ruta Otofte	0.3 *(0.54)*	1.8	IV	N2c	
*DF: Group II = 795; Group III = 567; Group IV = 477					

TABLE 3.1 (cont.)

Host	Disease Index mean (± SED)*	Doon Major Disease Index	Group test number	Isolates with indices substantially different from average	
				above	below
(c) *B. oleracea* cultivars					
var. *acephela* (marrowstem kale)					
Maris Kestrel	1.2 *(0.54)*	2.9	II		
Marrowstem	0.7 *(0.54)*	3.9	V		
var. *botrytis* (cauliflower)					
All The Year Round	0.2 *(0.54)*	3.9	V		
Mill Reef	0.5 *(0.54)*	3.9	V		
var. *capitata* (cabbage)					
Red Medium	1.5 *(0.54)*	2.9	II		
Cluseed Early	0.9 *(0.64)*	3.0	III		
Evesham	1.1 *(0.64)*	3.0	III		W9
var. *fimbriata* (kale)					
Debonair	0.7 *(0.54)*	2.9	II		
Vulcan	0.3 *(0.54)*	2.9	II		
var. *fructicosa* (thousand head kale)					
Canoon	1.1 *(0.54)*	2.9	II		C5
"	0.7 *(0.54)*	3.9	V	O8	C5,N2c
Thousand head	1.8 *(0.54)*	3.9	V	O8,O4b	N2c
var. *gemmifera* (Brussels sprout)					
Achilles	1.6 *(0.64)*	3.0	III		
Monitor	1.6 *(0.64)*	3.0	III		
Ormavon	1.3 *(0.64)*	3.0	III		
Palisade	2.2 *(0.64)*	3.0	III		N2c
var. *italica* (broccoli)					
Autumn spear	0.9 *(0.54)*	2.9	II		
Green Mountain	1.5 *(0.54)*	2.9	II		
var. *sylvestris* (cabbage, wild)					
DRS	1.0 *(0.54)*	2.9	II	O4b,O7a	N4a
DCT	0.7 *(0.54)*	2.9	II		

*DF: Group II = 795; Group III = 567; Group V = 510

TABLE 3.1 (cont.)

Host	Disease Index mean (± SED)*	Doon Major Disease Index	Group test number	Isolates with indices substantially different from average above	below
(d) Cruciferous crop and weed species					
Cultivated species:					
B. juncea (brown mustard)					
Trowse	0.3 *(0.54)*	1.8	IV		
B. carinata (black mustard)					
-	0.0 *(0.54)*	1.8	IV		
S. alba (brown mustard)					
Tilney	0.0 *(0.54)*	1.8	IV		
Raphanobrassica					
RB25/8	0.0 *(0.54)*	1.8	IV		
RB55	0.9 *(0.64)*	3.0	III		
R. sativus (radish)					
Nerys	0.9 *(0.54)*	3.0	III		
RS15	0.0 *(0.54)*	1.8	IV		
Weed species:					
Arabidopsis thaliana					
Thale cress	0.4 *(0.46)*	-	VII		
Capsella bursa-pastoris					
Shepherd's Purse	1.1 *(0.46)*	-	VII		
Cardaria draba					
Hoary cress	1.1 *(0.46)*	3.7	VI		
Lepidium sativum					
Garden cress	1.2 *(0.46)*	-	VII		
Raphanus raphanistrum					
Runch	0.2 *(0.46)*	3.7	VI		
Sinapsis arvensis					
Charlock	3.1 *(0.46)*	-	VII		
Sisymbrium officinale					
Hedge mustard	4.0 *(0.46)*	-	VII		
Thlaspi arvense					
Penny cress	2.0 *(0.46)*	-	VII		

TABLE 3.1 (cont.) (e) Non-cruciferous species

Family	Species	Crop	Common name/ cultivar	Mean Disease Index**
Chenopodiaceae	*Beta vulgaris* L. *rapa*	Fodder beet	Pajberg Rex	0
Compositae	*Lactuca sativa*	Lettuce	Webb's Wonderful	0
Cucurbitaceae	*Cucumis sativus*	Cucumber	Parisian Pickling	0
Fumariaceae	*Fumaria officinalis*	-	Fumitory	0
Geraniaceae	*Geranium zonale*	-	Geranium	0
Graminae	*Avena sativa*	Oats	Saladin	0
	Hordeum vulgare	Barley	Golden Promise	0
	Secale cereale	Rye	-	0
	Triticum aestivum	Wheat	Aquila	0
Leguminosae	*Phaseolus vulgaris*	Runner bean	Kelvedon Marvel	0
	Pisum sativum	Pea	Senator	0
	Vicia faba	Broad bean	Bunyard's Exhibition	0
Papaveraceae	*Papaver dubium*	-	Long-headed Poppy	1.8
	P. rhoeas	-	Corn Poppy	2.8
	P. somniferum	-	Opium Poppy	0.8

**Disease Index for Doon Major = 3.2

disease development. Different weed species showed a highly variable response (Table 3.1d): charlock and hedge mustard gave high average disease indices, while runch and thale cress show very little infection. Of the non-cruciferous hosts tested only members of the Papaveraceae show any disease development (Table 3.1e).

From the mean disease scores of each isolate on each group of hosts tested (Table 3.2), a few isolates gave slightly higher average disease indices (N4a, N2e and N2f) while others tended to give slightly lower average disease levels (W9, O4a and N5). However, most isolates tested behaved similarly. The variation in the ability of isolates to infect the individual hosts tested was generally small (Figure 3.1) although, as already indicated, there were interactions between isolates and hosts in some tests (Table 3.1). With *B. campestris* hosts three isolates, N2c, N2e and N6, all isolated from *B. napus* plants, gave above average infection on some cultivars of var. *rapifera* (Table 3.1a). With the *B. napus* hosts (Table 3.1b) the three *B. napus* isolates, N2c, N2e and N2f, caused above average infection on some cultivars while isolate O4a, from *B. oleracea*, caused below average infection on one swede cultivar. Two isolates collected from *B. oleracea*, O7a and O4b, showed above average infection on the *B. oleracea* hosts (Table 3.1c): four isolates caused below average infection on *B. oleracea* hosts, two originating from *B. napus*, N4a and N2c, one from *B. campestris*, C5, and one from *S. arvensis*, W9. Although statistically significant differences in disease levels occurred between isolates on certain hosts, only a few isolates evidenced large differences from the average (Figure 3.1). With respect to *B. campestris* (Figure 3.1a), cultivars Manchester Market and Green Top Scotch were highly resistant to all isolates except N2c, N2e and C5, while Findlay was infected to a greater or less extent by

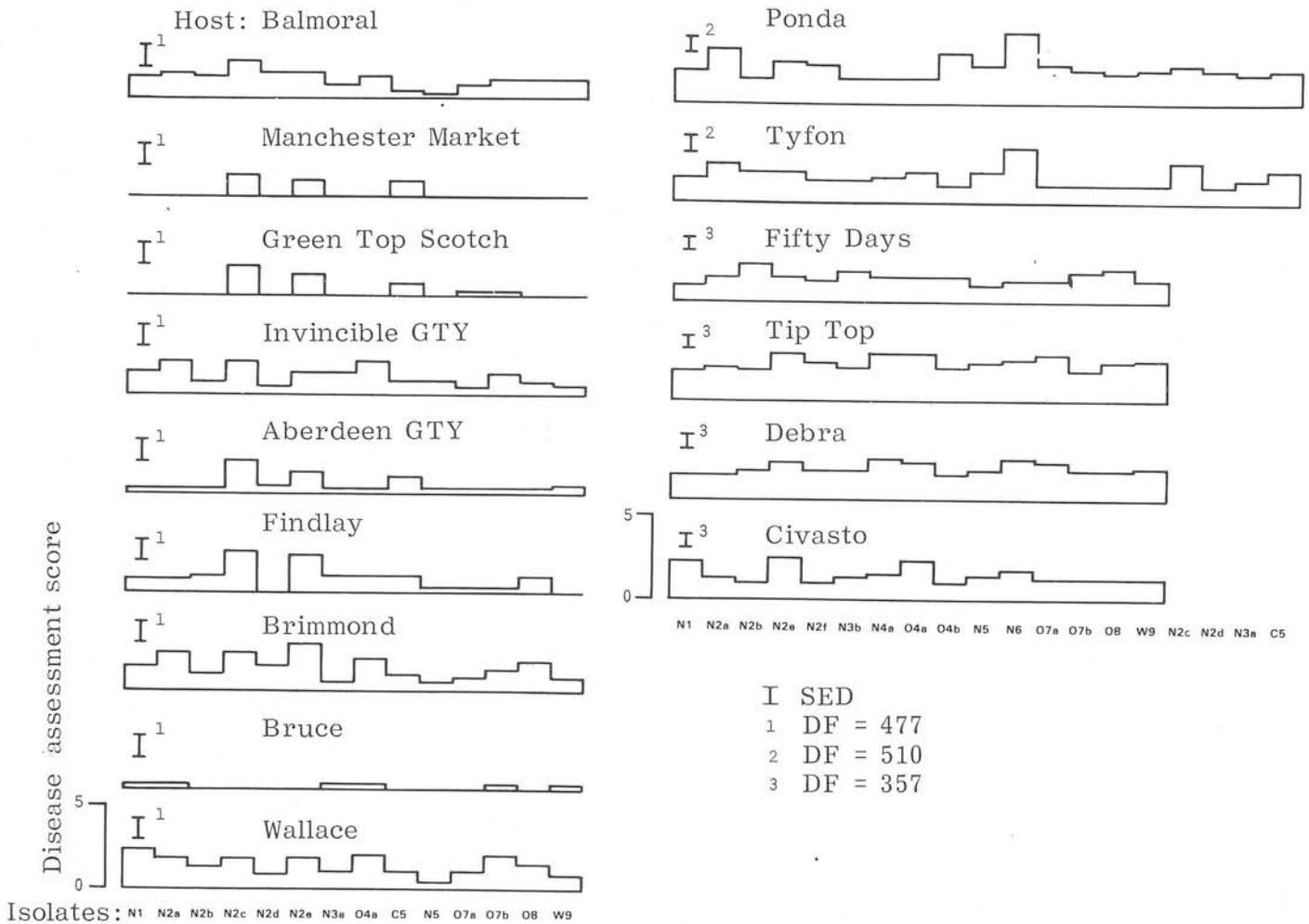


FIGURE 3.1: Disease indices recorded on different *Brassica* species for different isolates of *E. cruciferarum*. (a) *B. campestris* hosts.

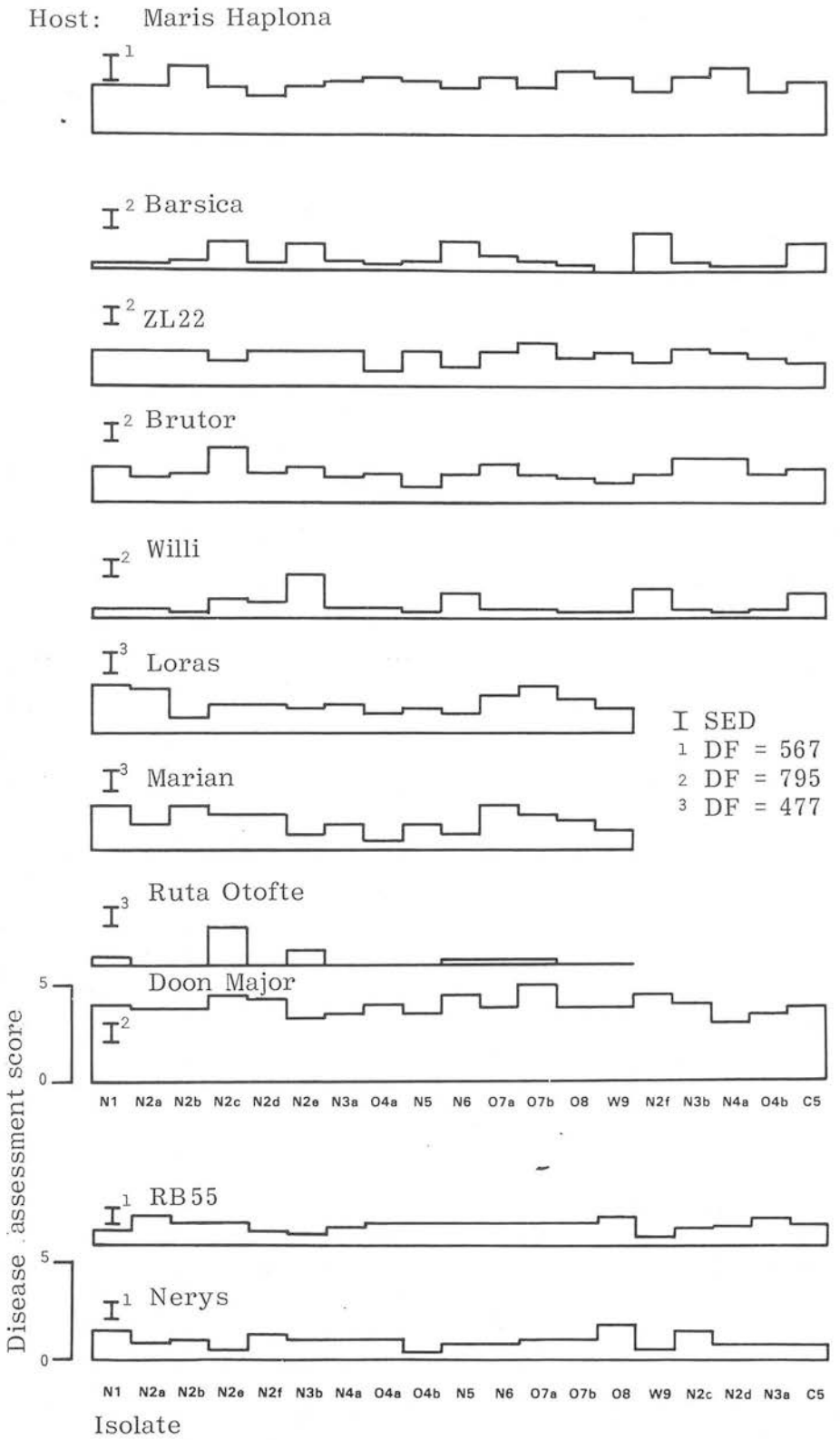


FIGURE 3.1 (cont.): (b) *B. napus* hosts, *Raphanobrassica* line RB55, and *R. sativus* cultivar Nerys.

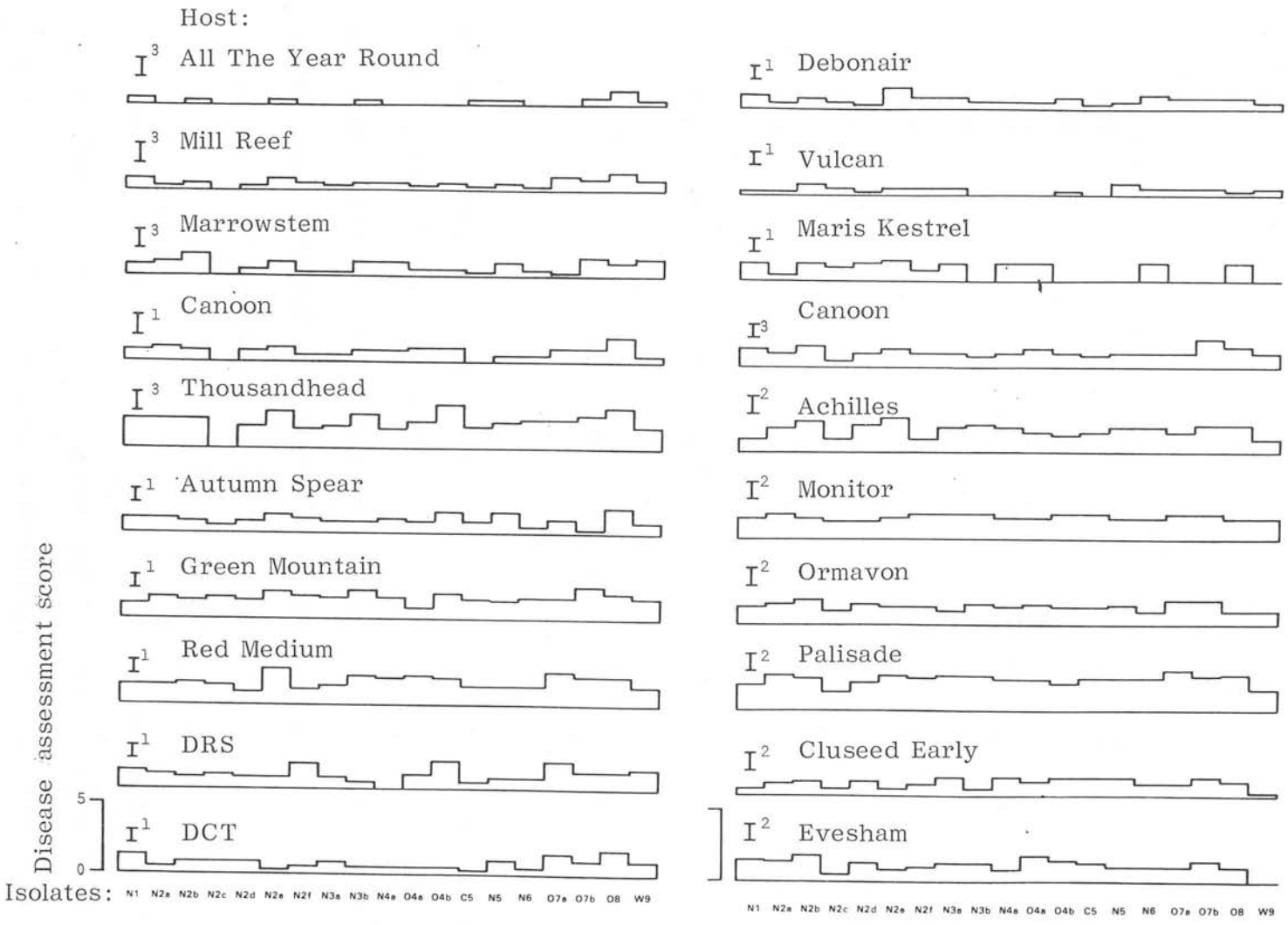


FIGURE 3.1 (cont.): (c) *B. oleracea* hosts.

I SED
1 DF = 795
2 DF = 567
3 DF = 510

TABLE 3.2: Mean disease indices for different isolates of *E. cruciferarum* in different group tests.

Isolate	Host group test I	II	III	IV	V	VI	VII	Mean
N1	1.1	1.2	1.5	0.8	1.6	1.5	1.7	1.3
N2a	1.3	1.0	1.7	0.5	1.8	1.4	-	1.3
N2b	1.0	1.1	2.0	0.7	1.7	1.5	1.7	1.4
N2c	2.2	1.2	1.3	0.9	1.4	-	1.7	1.5
N2d	0.8	1.0	1.6	0.7	1.4	-	2.4	1.3
N2e	1.9	1.5	1.6	0.6	1.7	1.7	1.7	1.5
N2f	-	1.2	1.5	-	1.4	1.3	2.2	1.5
N3a	0.9	1.1	1.7	0.5	1.4	-	2.0	1.3
N3b	-	1.2	1.7	-	1.5	1.4	-	1.5
N4a	-	1.1	1.7	-	1.4	1.6	2.2	1.6
O4a	1.3	0.9	1.7	0.3	1.4	1.8	-	1.2
O4b	-	1.2	1.5	-	1.9	1.3	-	1.5
N5	0.4	1.0	1.6	0.5	1.6	1.4	2.1	1.2
N6	-	1.2	1.6	0.4	2.0	1.7	2.0	1.5
O7a	0.8	1.4	1.8	0.6	1.5	1.5	-	1.3
O7b	1.1	1.4	1.8	0.7	1.5	1.5	-	1.3
O8	1.1	1.2	1.9	0.7	1.9	1.5	1.5	1.4
W9	0.8	1.1	1.2	0.3	1.5	1.5	-	1.1
C5	1.0	1.0	1.6	-	1.3	-	2.2	1.4
SED±	0.22	0.14	0.12	0.17	0.18	0.16	0.19	
(DF)	(477)	(795)	(567)	(477)	(510)	(357)	(215)	

all isolates except N2d and W9. Of the cultivars of *B. napus* (Figure 3.1b), W9 was the only isolate to show no development of infection on Barsica, which usually tended to show only moderate resistance. Eight isolates, including *B. napus* and *B. oleracea* isolates and W9 failed to develop mycelial growth on Ruta Otofte although others, notably N2e, did. The isolate N2c failed to develop on several *B. oleracea* hosts on which mycelial development occurred with other isolates (Figure 3.1c). One of those hosts was Maris Kestrel on which N3b also did not develop, while isolate N4a failed to develop on DRS. There was no

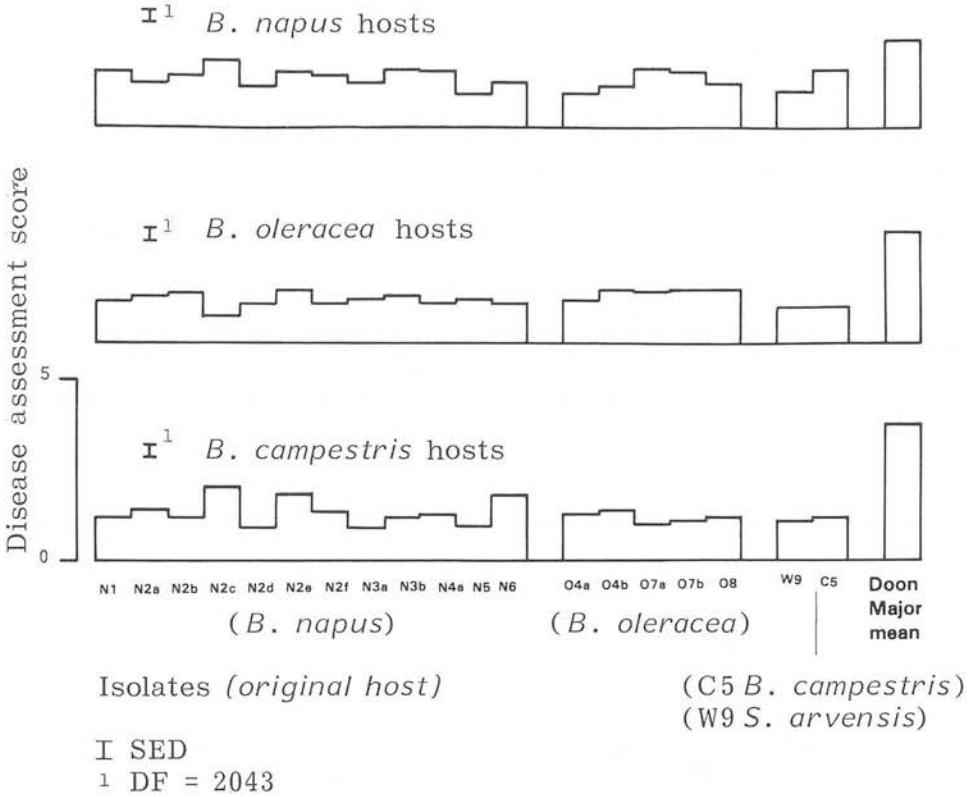
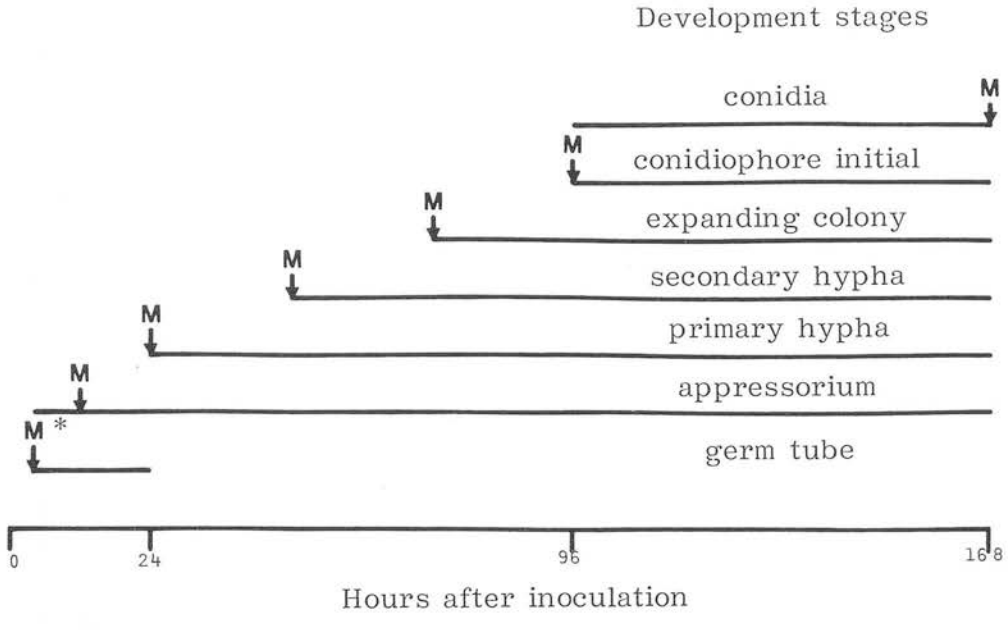


FIGURE 3.2: Mean disease indices for different isolates of *E. cruciferarum* on the *Brassica* crop species, *B. campestris*, *B. napus* and *B. oleracea*.

evidence of large interaction effects with other susceptible crop hosts (Table 3.1d and e).

The ability of the isolates to infect the main *Brassica* crop species is summarised in Figure 3.2, which gives the average responses of the three groups to the various isolates. There was no evidence of a clearly defined host specificity. However, with *B. napus* isolates, *B. napus* and *B. campestris* tended to show similar responses with isolate N2c giving the highest disease index, and N2d, N3a and N5 relatively low indices. On the other hand, isolate N2c gave a low average disease index on the *B. oleracea* group. With isolates from *B. oleracea*, the *B. napus* group gave a more variable response than other host groups and this was also the case in comparing isolate C5 with W9.



*M = maximum percentage shown

FIGURE 3.3: General pattern of development with time of isolates of *E. cruciferarum* on the cultivar Doon Major.

Experiment 3.b: *Development pattern of isolates*

Germination of spores of all isolates had begun by 4 hours after inoculation: by 8 hours over 60% and by 24 hours over 90% of conidia of each isolate had at least developed a germ tube. Figure 3.3 illustrates the sequence of development of colonies, with respect to the time when successive stages first appeared. At each stage, however, a proportion of infection sites failed to develop to the next stage.

From the analyses of variance of the results for each stage of development in relation to isolate and time, the two factors showed significant effects but with a significant interaction. The results of observations at different times are illustrated in Figure 3.4. No isolate appeared to be always the most advanced and, conversely, none was always the least developed. A small number of infection sites at any one stage of development may represent either a delayed development to that stage or a more rapid advance to the next stage of development.

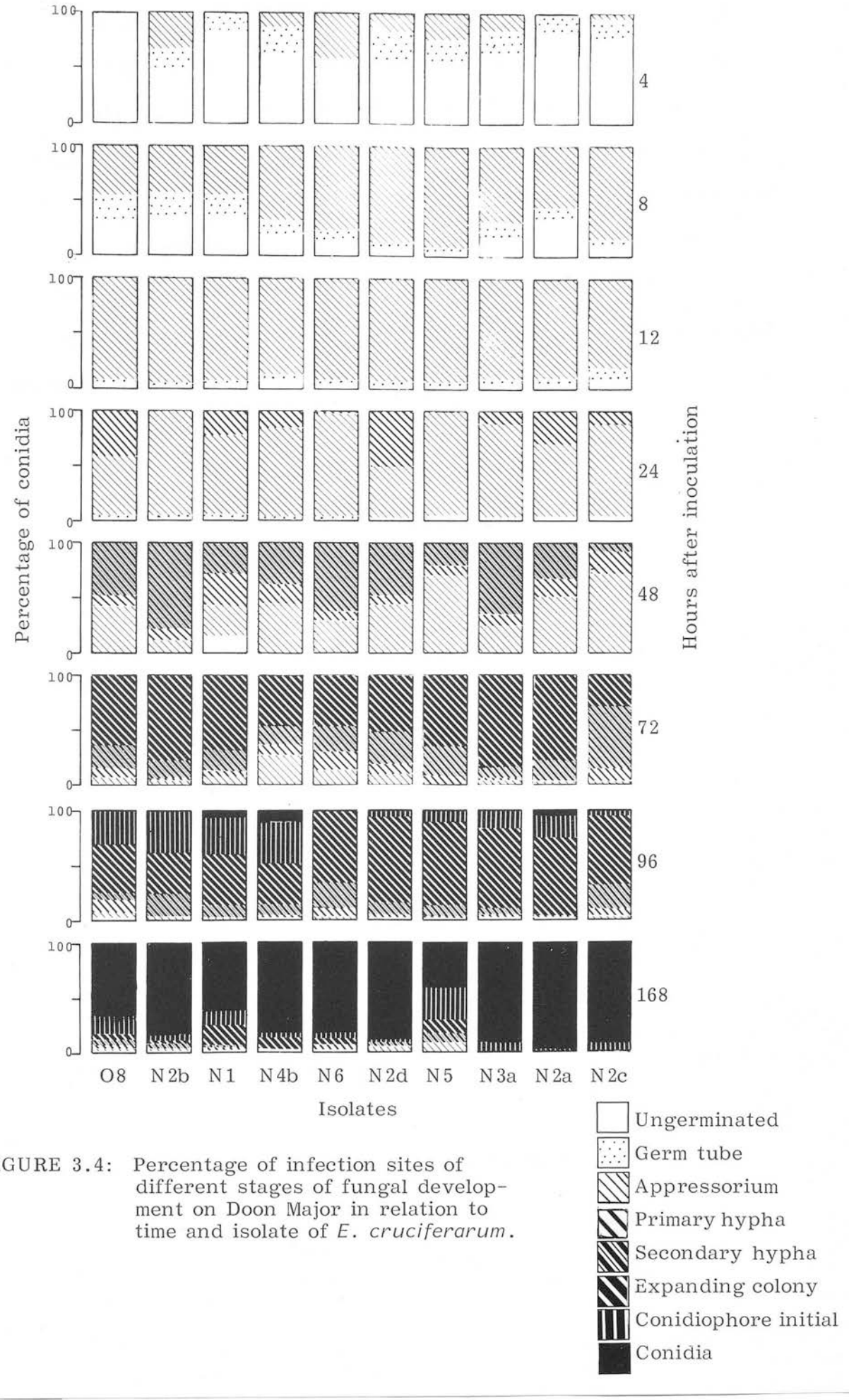


FIGURE 3.4: Percentage of infection sites of different stages of fungal development on Doon Major in relation to time and isolate of *E. cruciferarum*.

The extent and rate of development of the 10 isolates are illustrated in Figure 3.5. Germ tubes and appressoria developed at very similar rates for the 10 isolates and, after 48 hours, over 95% of conidia of each isolate had developed to or beyond these stages. However, once hyphal growth began, the rate of development to each stage varied more between isolates and, as development progressed, differences between isolates increased. Isolates which developed most rapidly to the primary hypha stage did not, however, necessarily show the fastest development to subsequent stages.

The variation in the extent of development between isolates at 96 and 168 hours can be seen in Figure 3.6: isolate N2a had the greatest percentage of colonies with conidia after 168 hours, although several other isolates were more advanced at 96 hours. At 168 hours, the extent of development showed a continuous variation between isolates from the most advanced which had 96% of colonies with conidia down to the least advanced isolate having only 42% of colonies with conidia. With the isolates which generally showed more advanced development, i.e. N2a, N3a and N2c, all infection units had developed to at least the conidiophore initial stage. In an intermediate group, comprising N2d, N2b, N4b and N6, a small proportion of infection sites showed no development beyond the primary hypha, secondary hypha or expanding colony stage. In the least developed group, O8, N1, and N5, a somewhat higher proportion of infection units appeared to have stopped at these stages, while the proportion of colonies with conidiophore initials was also higher than with the other isolates.

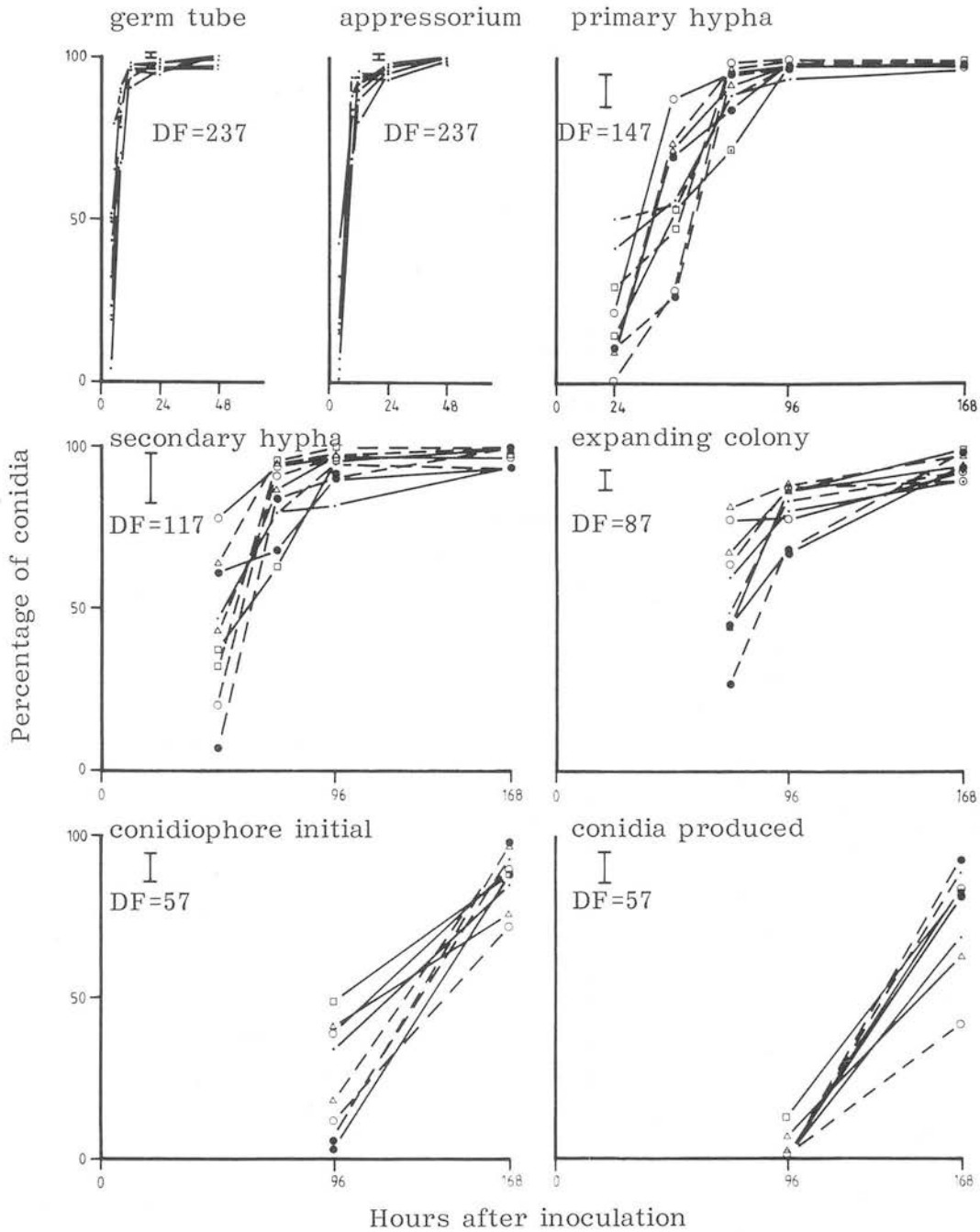


FIGURE 3.5: Development of different isolates of *E. cruciferarum* on Doon Major in relation to time after inoculation.

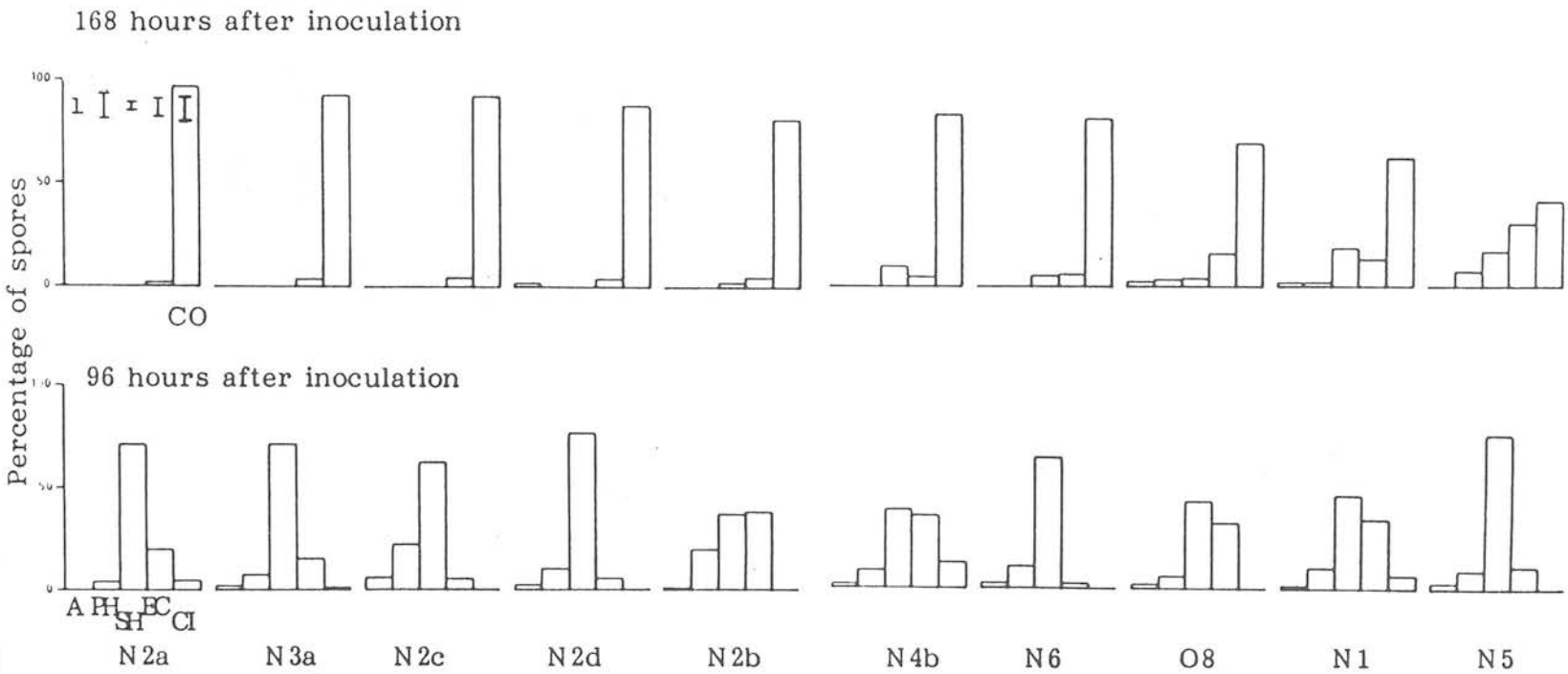


FIGURE 3.6: Percentage of infection sites at different stages of development at 96 and 168 hours after inoculation for different *E. cruciferarum* isolates on Doon Major.

Key:	Fungal Development	(DF)
A	Appressorium	147
PH	Primary hypha	117
SH	Secondary hypha	87
EC	Expanding colony	57
CI	Conidiophore initial	27
CO	Conidia produced	27

Experiment 3.c: *Conidial production of isolates*

In checking the inoculum dose levels for the different isolates, it was found that the mean numbers of conidia of isolates N2a, N2b and N5 deposited on leaf discs were similar, although numbers for N2b were slightly higher (Table 3.3). However, the spore numbers of isolate O8 were substantially lower than those of any of the others. There was little difference between isolates in the percentage germination of conidia, the means ranging from 87 to 91.

TABLE 3.3: Estimated mean number of deposited conidia per leaf disc when inoculated with four *E. cruciferarum* isolates. (*Area of leaf disc 354.3 mm^2.)

Isolates	N2a	N2b	N5	O8	SED± (DF = 392)
Total spore number/ leaf disc*	1338	1569	1301	171	66.2

Conidia were first produced from colonies 4 days after inoculation and the overall average number per leaf disc per day increased steadily from 4 to 10 days, then showed a decline before reaching a peak at 13 days followed by a further decline at 14 days, when readings ended (Figure 3.7).

Significant differences in conidial production were associated with isolates, although from 4 to 7 days after inoculation the numbers of conidia produced per leaf disc by each isolate were similar, and from 4 to 9 days no significant differences between isolates were found in the number of spores produced per colony (number of spores per leaf disc divided by numbers of spores in the inoculum). Over the period of observation no isolate showed consistently a higher daily rate of spore production per leaf disc (Figure 3.8a), but isolate N5 showed

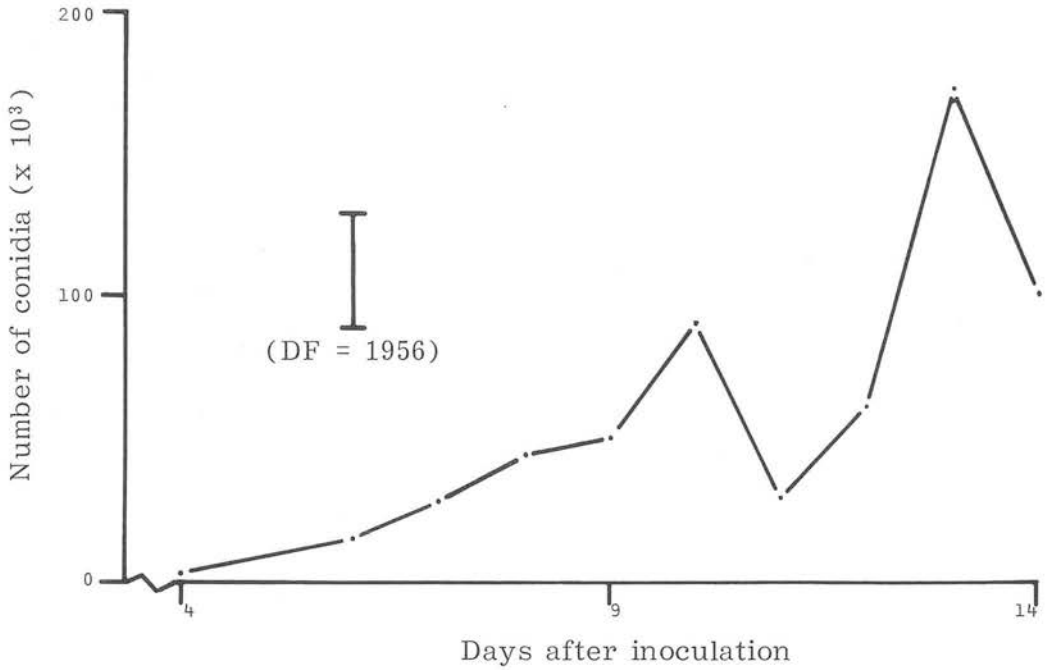


FIGURE 3.7: Mean daily rate of production of conidia per leaf disc on the host Doon Major from 4 to 14 days after inoculation with four *E. cruciferarum* isolates.

the highest cumulative total (Figure 3.8b). Isolate O8, despite its low inoculum density, showed a similar total spore production to isolate N2a and a higher level than isolate N2b: spore production rate per colony was markedly higher for isolate O8 than for any other colony (Figure 3.9). The total number of conidia produced per colony for isolates O8, N5, N2a and N2b were 3257, 682, 426 and 261 respectively.

Experiment 3.d: *Conidial size of isolates*

Slight but highly significant differences were found in the length, breadth and ratio of length to breadth of conidia of different isolates (Figure 3.10). The mean length of conidia of isolates ranged from 41.9 to 44.9 µm, whilst the mean breadth varied from 13.9 to 19.5 µm.

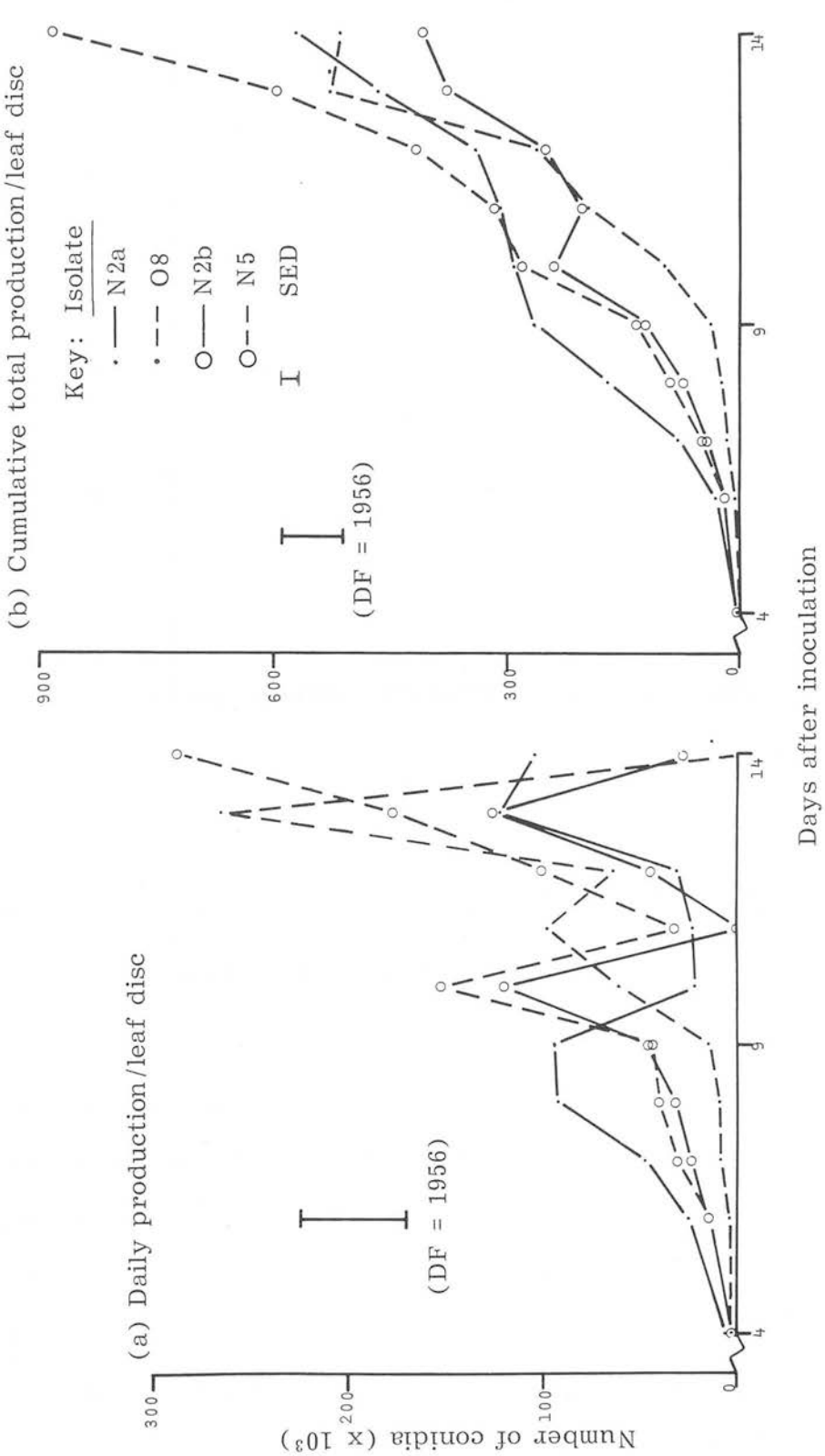


FIGURE 3.8: Daily production and cumulative total production of conidia per leaf disc of Doon Major by four *E. cruciferarum* isolates over the period 4-14 days after inoculation.

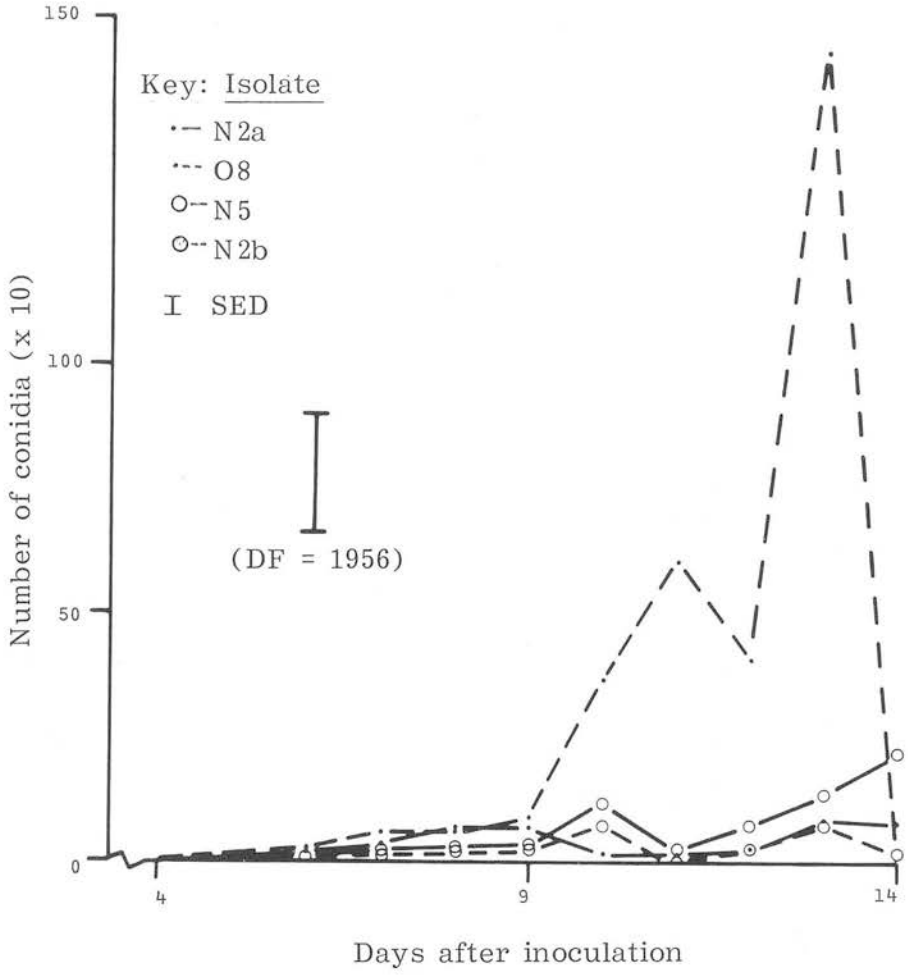


FIGURE 3.9: Daily rate of production of conidia/colony of four isolates of *E. cruciferarum* on the host Doon Major from 4 to 14 days after inoculation.

Isolates N2b and N6 produced conidia which were longer than the others and conidia of N2a were relatively short (Figure 3.10a), while isolates O4a and N2f were narrower and wider respectively than the other isolates (Figure 3.10b). The ratios of length to breadth for each isolate varied from 2.2 to 3.1 (Figure 3.10c), isolates N5 and O4a giving relatively high ratios and N2a and N2f low. In comparing Figures 3.10b and c, it appeared that where conidia were broader the ratio of length to breadth tended to decline.

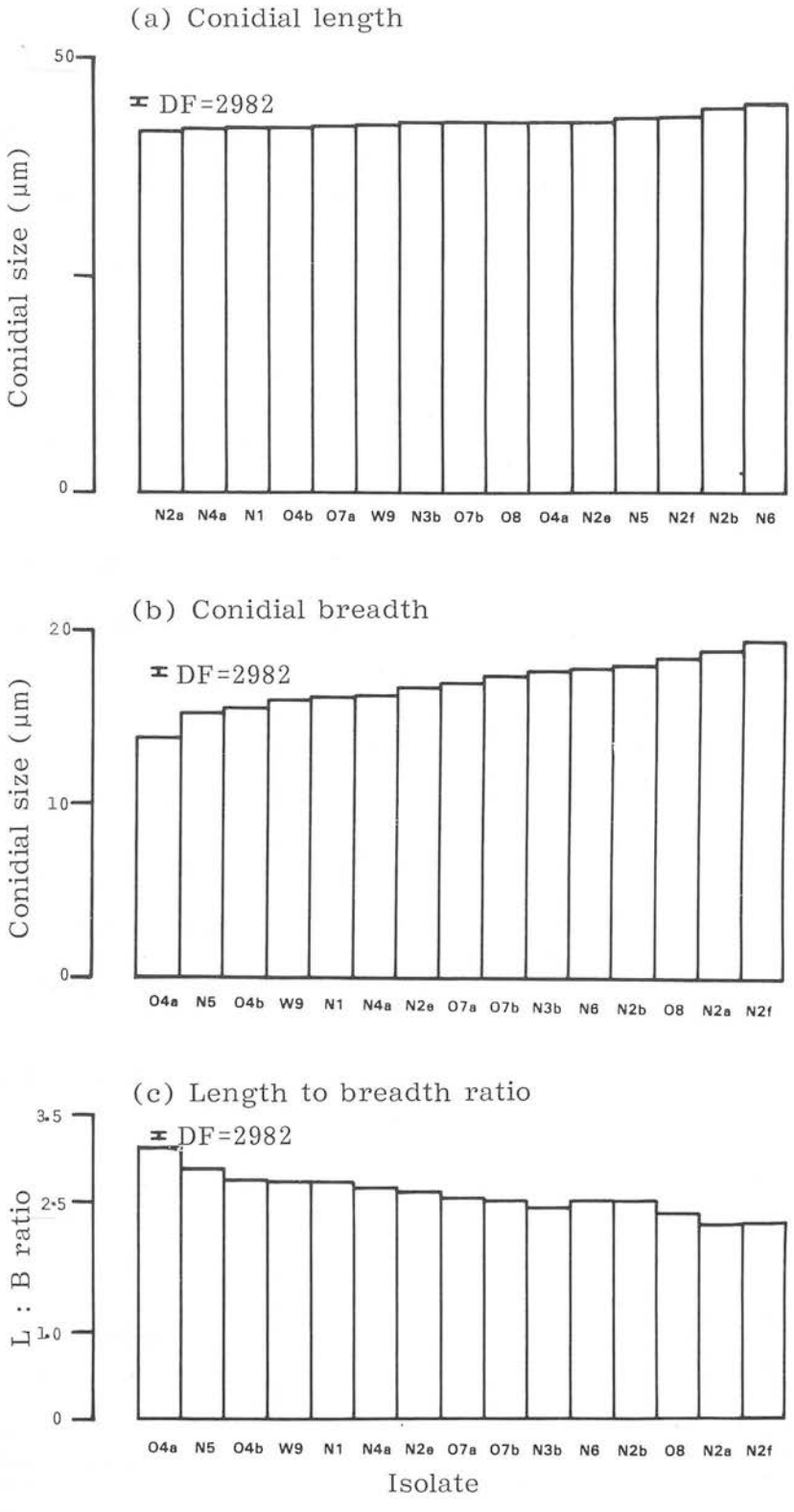


FIGURE 3.10: Mean conidial dimensions and length to breadth ratio of different isolates of *E. cruciferarum*.

DISCUSSION

The results of leaf disc inoculation studies, using several isolates of *E. cruciferarum* from various hosts and localities on a wide range of plants, indicated that the host range of the species is confined to members of the family Cruciferae and Papaveraceae. This is in keeping with the findings of Junell (1967) when she segregated *E. cruciferarum* from *E. communis*. Of the other families tested, none were found to become infected, although two of these families, Fumariaceae and Geraniaceae, had been listed as hosts by Boesewinkel (1979): the present results must bring into question his suggestion of a wider host range than that delimited by Junell.

When Junell described *E. cruciferarum*, she gave the size of conidia as 12-16 x 30-40 µm. Since 1967, many other workers have examined and measured conidia of *E. cruciferarum* collected from different cruciferous species and, generally, larger dimensions have been reported (Table 3.4).

TABLE 3.4: Dimensions of conidia of *E. cruciferarum* collected from cruciferous species as reported by different workers.

Source	Conidial size (µm) breadth	length
Junell (1967)	12.0 - 16.0	30.0 - 40.0
Sankhla *et al*. (1967)	10.5 - 19.5	24.5 - 42.0
Sharma (1977)	13.1 - 16.9	30.0 - 41.2
Boesewinkel (1979)	11.0 - 17.5	27.0 - 55.0
Boesewinkel (1980)	16.2 - 17.5	42.0 - 50.0
Saharan & Kaushik (1981)	8.3 - 20.8	20.8 - 45.8
Boesewinkel (1981)	15.0 - 18.0	36.0 - 52.0
Present study	13.9 - 19.5	41.9 - 44.9

The ranges in breadth and length of conidia reported may be seen to vary for different studies, while work by Sharma (1977), Saharan and Kaushik (1981) and Boesewinkel (1980) showed variation associated with the hosts on which the conidia were produced. The results here show some variation among isolates from different sources, but no consistent size feature linked with particular host species.

Conidial size can be affected by external influences, for example leaf surface, humidity and ambient temperature (Yarwood, 1957; Staveley and Hanson, 1966). Conidia of *E. polygoni* from red clover averaged 18 x 30 µm, 20 x 36 µm and 18 x 33 µm when collected from the field, greenhouse and detached leaves floated on sucrose solution respectively (Yarwood, 1957). Of the previous research workers listed in Table 3.4, none reported their experimental methods such as incubation conditions, colony ages, number measured or conidia retrieval technique. It would appear that conidia were collected in all but one study from naturally infected plants in the field. Staveley and Hanson (1966), in considering the use of herbarium material, observed that dried conidia are generally smaller than fresh samples, and the relatively small dimensions of conidia recorded by Junell (1967) may be due to her use of herbarium specimens for assessments.

In comparing the behaviour of the isolates used in this study, there was little evidence of marked qualitative differences at a level which would suggest the existence of *formae speciales*. Thus, cross infections between isolates from different host species or genera readily occurred and the level of resistance of a particular host species tended to be reflected over the whole range of isolates. Any occurrence of specific interactions with large effects occurred only occasionally within host species at a cultivar level: thus, cultivars such as Green Top

Scotch and Findlay (*B. campestris*) and Ruta Otofte (*B. napus*) showed no disease development with isolate N2d, whereas N2c gave a relatively high disease index on these cultivars. This might indicate the occurrence of major gene effects, but only at a low frequency. However, genetical analyses (Walker and Williams, 1965; Brain and Whittingdon, 1980) of *B. oleracea* and *B. napus* have shown there to be no major genes for resistance, a feature of nearly all recorded host-parasite specific interactions (Sidhu, 1975). In general, most isolates were able to infect, at least to some degree, most cruciferous hosts tested. In many biotrophic fungi, host specific pathogenicity is usually expressed at a high level and easily identified (Scott *et al.*, 1978), but this does not seem to be the case with *E. cruciferarum*. Most of the variation in isolate-host responses tended to be quantitative rather than qualitative in nature. This contrasts with, for example, *Erysiphe graminis* and barley where clear-cut qualitative variation in pathogenicity of isolates on different hosts occurs frequently (Abbott, 1983). The lack of specificity in *E. cruciferarum* may reflect the outbreeding characteristic of members of the host family Cruciferae, which are closely allied with much genetic affinity between taxa (U, 1935). Moreover, the lack of any strong major genes for resistance gives rise to low selection pressure for increased virulence in the pathogen: according to Van der Plank (1968), where hosts tend to exhibit horizontal resistance only, isolate behaviour deviates only slightly around the average.

With respect to quantitative variation, there was some degree of host specificity with, for example, isolates from *B. napus* and *B. oleracea* having a slightly greater affinity for their respective original host species, as expressed by their disease indices: in addition, at a cultivar level some isolates gave more infection on a particular host

than others. In barley mildew this type of variation has been related to differences in 'background' genes for resistance against which the pathogen may have more or less virulence (Wolfe and Schwarzbach, 1978).

In considering the average disease indices produced by different isolates over the range of hosts tested, significant differences were demonstrated. This evidence of quantitative variation in the general pathogenicity of isolates may have a genetic basis, although it may reflect, in part, the balance of the particular hosts used in the study and still be specific rather than general in nature. Moreover, the possibility of the conditions linked with the particular experiment which would not be reproducible cannot be discounted. Thus Doon Major, which was used in all tests, showed a variation in disease indices from one test to another (Table 3.1), while Canoon showed slightly different response patterns to different isolation in the two tests in which it was used (Figure 3.1c).

The results of studies on the development pattern of 10 isolates on the susceptible host, Doon Major, showed that colony development of all isolates progressed rapidly and, by 96 hours, conidiophore development was in progress with conidia initiated by colonies of several. Vegetative development of the isolates varied with time after inoculation but there was an inconsistent pattern of growth. Moreover, by 168 hours, the numbers of infection units which had reached or passed the expanding colony stage were similar between the 10 isolates. Quantitative variation in colony growth, from 3 to 20 days after inoculation, has previously been observed (Epton, pers. comm.), not only between isolates of *E. cruciferarum* but between conidia from a single pustule. Epton found growth erratic with no consistent pattern to

indicate inherent differences between isolates: variation was, therefore, considered unlikely to be determined by genetic factors. In the present studies, there was also nothing to suggest large intrinsic differences of a clearly defined nature between isolates in their infection behaviour.

The levels of conidial production of four of the isolates which had been shown to give varying rates of colony development on the susceptible host Doon Major were assessed: at 168 hours after inoculation 97, 82, 70 and 41% of isolates N2a, N2b, O8 and N5 respectively were at the sporulating phase, but no obvious relationship was evident between the rate of isolate development and early production of conidia, either per leaf disc or per colony. However up to 6 days after inoculation, when studies of development ended, levels of conidial production were very low with all isolates. The results suggested that conidia were produced from about 4 days after inoculation as indicated by Purnell (1971) and that the maximum daily rate of conidia production occurred at about 13-14 days after inoculation, although observations were discontinued at 14 days.

The total production of conidia per leaf disc was inversely related to the numbers of conidia inoculated onto each leaf disc for the three isolates N5, N2a and N2b while isolate O8 did not show the lowest spore production per leaf disc despite having a very low inoculum density in this experiment. In considering spore production per colony there was an obvious reduction in sporulating capacity of colonies with increasing inoculum density which may be related to inter-colony competition. Working with *Uromyces phaseoli*, Davison and Vaughan (1964) observed that pustule size decreased with increasing levels of inoculum. Furthermore, studies on conidia production by *E. graminis* (Shaner, 1973)

have shown that conidia production decreases with decreasing colony size: as competition for host substrate increases colony growth decreases and sporulation capacity declines. The deleterious affect of high inoculum densities on colony growth on a susceptible host, as found in this experiment, may partly explain why in experiments on adult plant resistance of spring barley to *E. graminis* (Hwang and Heitefuss, 1982) susceptible and resistant cultivars could not be distinguished when high inoculum levels were used.

Regardless of inoculation level, conidial production by colonies of all isolates were very similar during the first 9 days after inoculation and it was only after this time that an effect of inoculum density on conidia production can be seen (Figure 3.10): the results suggest that with viable spore numbers of greater than about $300/cm^2$ inter-colony competition is marked compared with an inoculum density of about $50/cm^2$.

The lack of variation in early conidial production between different isolates would suggest that for those studied there was no variation in period of latency to account for any difference in infection rate of significance in an epidemic context (Van der Plank, 1968). The patterns of conidial production tend to show a relatively slow build up in rate. This is in keeping with observations (Lennard, unpublished data) that rate of conidia production of *E. cruciferarum* on a susceptible cultivar is relatively slow compared with *E. graminis* on wheat, barley and oats. Moreover, Asher and Thomas (1984) found that where variations in the rate of conidial production of *E. graminis* on different barley hosts occurred, they became obvious only during peak production periods. Working with two isolates of *E. graminis* f. sp. *tritici*, Hyde and Colhoun (1975) found a wide variation in their sporulation capacity on the susceptible cultivar Joss Cambier: over six experiments the percentage

of colonies reaching conidial group 4 (more than 40 conidial chains), by 7 days after inoculation, ranged from 60 to 94 with isolate W9/30 and, from 70 to 89 with W71/178.

In conclusion, the host range of *E. cruciferarum* is confined to members of the families Cruciferae and Papaveraceae. Within the Cruciferae, the family most extensively studied, variation in pathogenicity between isolates tended to be quantitative rather than qualitative, with only a few instances of clearly defined host specificity. Within the range of hosts tested, however, a wide range of differences in disease response was evident. These two aspects, variation in host resistance and the evidence for host specific interaction among isolates, are investigated further in the next section.

4. STUDIES ON FUNGAL DEVELOPMENT AND PLANT TISSUE RESPONSE

INTRODUCTION

From the literature, there appears to be a range of levels of resistance expressed by different members of the family Cruciferae in response to infection by *E. cruciferarum*. Resistance varies, not only between different genera, but also within genera, at species, sub-species and cultivar levels (Narain and Siddiqui, 1965; Purnell, 1971; Brain, 1978; Dixon, 1977; Anon, 1978). The factors involved in the resistance of cruciferous hosts to infection by *E. cruciferarum* has, however, been little studied. Working with swede cultivars (*B. napus*), Brain and Whittingdon (1980) demonstrated that resistance is largely polygenic, and expressed as a restriction of colony extension and re-infection (Brain, 1978). Cell necrosis has been implicated in the resistance of cabbage (*B. oleracea*) to *E. cruciferarum* (Walker and Williams, 1965).

Host resistance to other powdery mildew fungi has been studied in great detail. For example, important components of generalised host resistance to attempted penetration include the formation of cytoplasmic aggregates and epidermal cell wall thickenings such as papillae (Bushnell and Berquist, 1975; Aist, 1976; Ride and Pearce, 1979; Sherwood and Vance, 1980). After penetration of the host by a powdery mildew fungus, a haustorium is formed in the epidermal cell of a susceptible host. Formation of a functional haustorium is crucial to the development of a compatible pathogen-host relationship (Ellingboe, 1972). In certain barley cultivars, resistance retarded haustorial development, reduced the size of haustoria and after 5 days most haustoria were either distorted or degenerate (Masri and Ellingboe, 1966).

Host cell necrosis has been associated with reduced fungal growth, either by limiting access of the biotrophic parasite to living host cells or by releasing substances inhibitory or lethal to the pathogen (Bushnell, 1982). The term 'hypersensitivity' is used when early cell necrosis is associated with resistance. Hypersensitivity can take one of two forms (Brown, Shipton and White, 1966): the most common reaction is indeterminant hypersensitivity which is characterised by continuing slow fungal growth and progressive host cell necrosis: by contrast, with determinant hypersensitivity the host cells die and fungal growth is halted soon after the primary haustorium is produced (Bushnell, 1981). A causal relationship between hypersensitivity and resistance has been widely accepted but there are cases in which the two do not seem related. Brown *et al.* (1966) were unable to find any consistent relationship between colony size of *Puccinia graminis* f. sp. *tritici* on wheat leaves and the rate of increase of collapsed tissue. They concluded that hypersensitivity was not the cause of resistance, but a consequence of infection in the resistant hosts used.

Host resistance is not only expressed qualitatively but also affects fungal development quantitatively: resistance of cereal crops to *E. graminis* has been shown to reduce the number of successful primary penetrations (Carver and Carr, 1977), delay colony development (Carver and Carr, 1978) and reduce sporulation (Shaner, 1973; Asher and Thomas, 1984).

In 1972, Ellingboe concluded from his studies with *E. graminis* that various genotypes for incompatibility present a series of hurdles for the parasite. A certain portion of the parasite units get over each hurdle but the portion stopped by each hurdle and the time after inoculation when the interactions between host and parasite affect the ontogeny

of the parasite are unique to each gene pair. Working with *E. graminis* f. sp. *tritici* and *E. graminis* f. sp. *hordei*, Ellingboe was able to define the points during the early stages of infection at which resistance was expressed: he emphasised the value of observations made on the early interactions of particular pathogen-host genotype combinations in the establishment of cause-and-effect relationships. The well documented accounts of resistance to *E. graminis* infection contrasts with the scant knowledge of the basis of resistance to infection by *E. cruciferarum*. In the previous experimental study (Section 3), variation in the levels of disease development were found on different host plants and, in some instances, variation was associated with different isolates on the same host. In this section of the experimental studies, detailed accounts are given of the results of microscopic studies of the progress of infection by isolates of *E. cruciferarum* on members of the family Cruciferae which varied in their degree of susceptibility. Four studies are considered as follows:

4.a Disease development studies with *E. cruciferarum*.

4.b Progress of colony development of *E. cruciferarum*.

4.c Progress and extent of colony development of *E. cruciferarum*.

4.d Haustorium formation by *E. cruciferarum*.

MATERIALS AND METHODS

In each of the four experiments plants were grown in a glasshouse and inoculations carried out 11 weeks after sowing according to the methods described in Section 2. The inoculum of each isolate was prepared on the cultivar Doon Major and leaf discs of the host plants in tests were inoculated, unless otherwise indicated, in a large settling tower before inoculation. In Experiments 4a to 4c, 50 conidia were assessed on each of four or 10 (Experiment 4a) replicates of each host/isolate combination and, where appropriate, at every time interval. Only necrotic cells and/or fluorescent sites below fungal structures were counted.

Experiment 4.a

Three *B. napus* cultivars, Doon Major, Ruta Otofte and Barsica, and a line of *Raphanobrassica*, RB25/8, along with three pathogen isolates, N2a, N2e and N3a, were used. Infection levels were assessed 5 days after inoculation using the 0-5 Disease assessment scale (Figure 2.2). Immediately after assessment, leaf discs were prepared for observation with a Leitz Ortholux II compound microscope which combined incident light fluorescence (exciter filter, UGI, 2 x 2 mm; suppression filter, K430; dichroic mirror, TK400; 200W mercury light source) with alternative or simultaneous object observation in transmitted bright field. Leaf discs were immersed in an ethanol/chloroform/trichloroacetic acid (TCA) solution (75 : 25 + 0.15% TCA) and left to clear overnight (Wolf and Fric, 1981). Following this, leaf discs were transferred to a solution of 0.1% aniline blue in 0.1 MK_2HPO_4 buffer (pH 9.2) for 24 hours (Shimomura and Dijkstra, 1975). Prior to examination, discs were

counterstained for fungal structures in 0.1% trypan blue in glacial acetic acid/distilled water (45 : 55) for 2 to 5 minutes, rinsed in distilled water and then mounted in the aniline blue solution. Uninoculated control leaf discs were also examined, following treatment by the method described above.

Spore germination rates were always very high and ungerminated and closely grouped conidia were not included in observations. The number of necrotic cells which were observed below each colony from a single conidium were counted. Necrotic cells were easily identified as they absorbed aniline blue and fluoresced when under ultra-violet light (Plate 4.1). The number of fluorescent sites (Plate 4.2) observed below each colony were also counted. Sites of fluorescence were found either associated with apparently normal cells (Plate 4.2) or within necrotic cells (Plate 4.3).

Experiment 4.b

Assessments of fungal development on five hosts, cultivars Doon Major and Barsica of *B. napus*, and lines RS15 of *R. sativus*, RB25/8 of *Raphanobrassica* and BC82 of *B. carinata*, inoculated with isolates N2e or N4a of *E. cruciferarum*, were made 2, 4, 8, 12, 24, 48, 72 and 96 hours after inoculation. At each assessment time, leaf discs were cleared by fuming with chlorine gas until bleached (Janes, 1962). Following neutralisation for 4-5 minutes with ammonia vapour, discs were stained with aniline blue and trypan blue as in the previous experiment. Fungal growth was assessed using the Fungal development scale (Section 2.d) and the numbers of necrotic cells and fluorescent sites found beneath colonies counted. Ungerminated conidia were not included in the observations after the 24-hour stage and, at 96 hours, observations

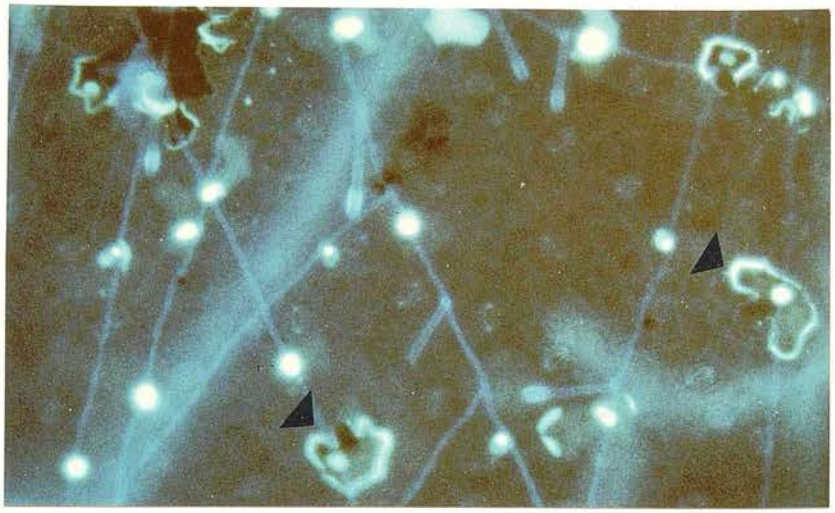


PLATE 4.1: Fluorescence of necrotic epidermal cells (stained with aniline blue, x 25).

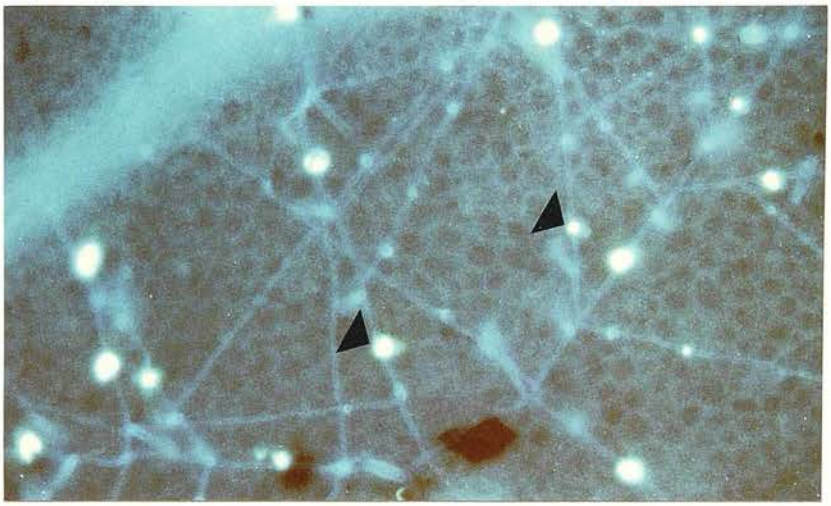


PLATE 4.2: Fluorescence at sites of penetration (stained with aniline blue, x 25).

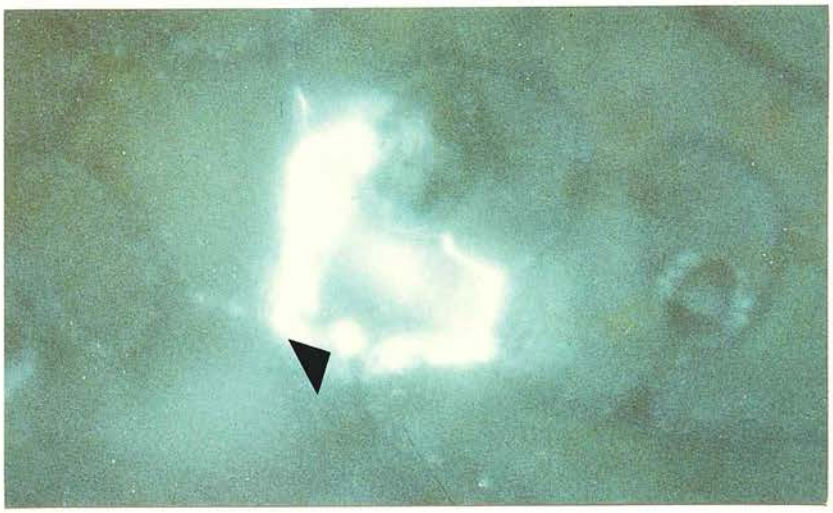


PLATE 4.3: Fluorescence at site of penetration and of necrotic epidermal cell (stained with aniline blue, x 100).

on leaf discs of control plants were made as a check on the appearance of uninoculated leaf discs.

Experiment 4.c

Using as hosts cultivars Doon Major (*B. napus*), Vobra (*B. campestris*), Cluseed Early and Achilles (*B. oleracea*) with isolates N2e, N4a and O4a, assessments of infection development on leaf discs were made 4, 8, 12, 18, 24, 48, 72 and 144 hours after inoculation. In this experiment, the stage of fungal development, the extent of branching of hyphae, the lengths of hyphae, and the numbers of necrotic cells, fluorescent sites and conidiophores were assessed. Leaf discs at each assessment time were fixed and cleared overnight in 95% alcohol before staining with aniline blue/trypan blue as described in Experiment 4.a. Uninoculated discs after 144 hours incubation were also examined.

Fungal growth was assessed using the Fungal development scale (Section 2.d). As also described earlier, emerging primary (1°) hyphae can be identified, i.e. 1°A, 1°B, 1°C, 1°D, 1°X (Figure 2.4). In an attempt to characterise the effects of host reactions on mycelial development, the length of each primary hypha was measured on each colony, as well as the lengths of associated secondary (2°) and tertiary (3°) hyphae.

Following germination, the numbers of necrotic cells were assessed. Numbers per colony were assessed at the germ tube or appressorium stage but at the primary hypha stage and subsequent stages the five initial primary hyphae (A, B, C, D or X) were treated individually: the number of necrotic cells associated with any one primary hypha was the sum of all necrotic cells found beneath the initial hypha and beneath the derived secondary and tertiary hyphae where present.

Fluorescent sites were categorised into small, < 7 µm (Plates 4.4 and 4.6), or large, ≥ 7 µm (Plates 4.5 and 4.6) and the numbers of each per colony were recorded in the same way as the necrotic cell assessments, at the germ tube, appressorium and primary hypha development stages. The numbers of conidiophores on each of the primary hyphae were recorded and, as with the necrotic cell assessments, the number of conidiophores attributed to any one primary hypha was the sum of those found on that primary hypha and associated secondary and tertiary hyphae.

Experiment 3.d

The cultivars Doon Major and Magres (*B. napus*), Vobra (*B. campestris*) and Lunet (*B. oleracea*), together with isolates N2e, O4a and O7, were used in this experiment. After incubation for 7 or 14 days, leaf discs were immersed in 95% alcohol and left to clear overnight, the discs were then mounted in 0.01% trypan blue in lactophenol and examined using a light microscope.

Assessments were made, 7 and 14 days after inoculation, of the numbers of haustoria which appeared normal (Plate 4.7), and of haustoria which appeared abnormal, i.e. collapsed or degenerate (Plates 4.8 and 4.9). In addition, the numbers of necrotic cells were recorded. Twenty colonies on each of three replicates of each host/isolate combination were assessed at each time interval. Clusters of conidia were ignored and each single colony was assessed by centralising in the field of view (x 250) the initial germinated conidium, and counting all haustoria and necrotic cells within the field of view.

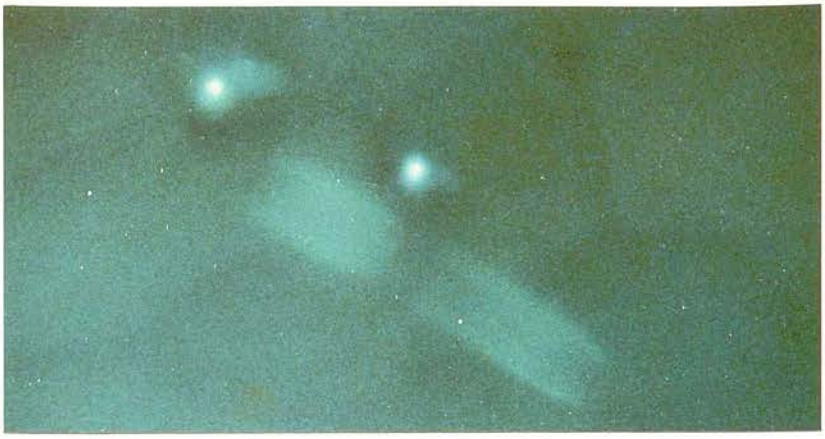


PLATE 4.4: Fluorescence (<7 µm diameter) at site of penetration (stained with aniline blue, x 100).

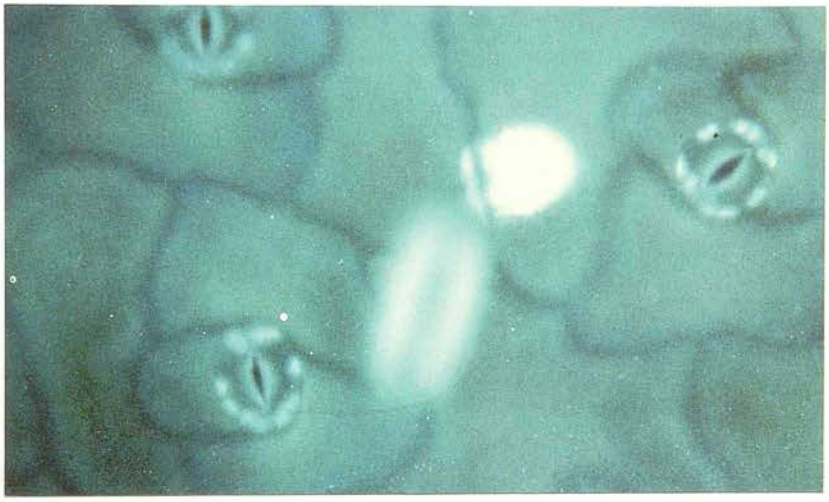


PLATE 4.5: Fluorescence (>7 µm diameter) at site of penetration (stained with aniline blue, x 100).

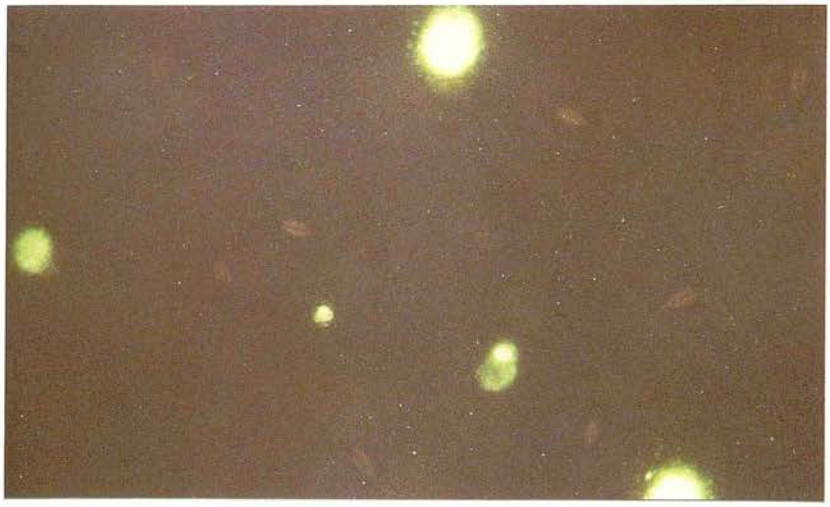


PLATE 4.6: Fluorescence at sites of penetration (aniline blue stain, yellow filter, x 62.5).

PLATE 4.7: Leaf surface view of haustorium of *E. cruciferarum* (x 100).

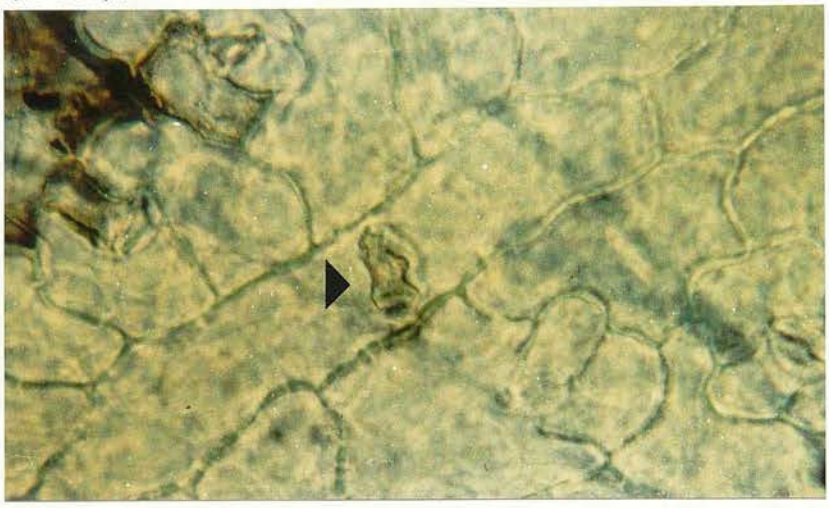


PLATE 4.8: Leaf surface view of collapsed haustorium of *E. cruciferarum* (x 100).

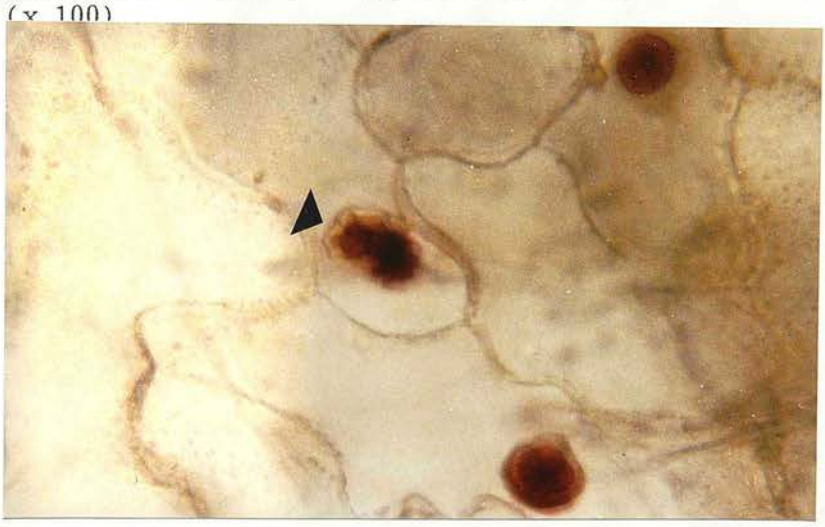


PLATE 4.9: Leaf surface view of degenerate haustorium of *E. cruciferarum* (x 100).

RESULTS

Experiment 4.a: *Disease development*

The analyses of the data for assessments of disease development, number of fluorescent sites/colony and number of necrotic cells/colony in relation to host and isolate showed that host had a significant effect in all three assessments: isolate had a significant influence only on number of fluorescent sites, although in this instance there was a significant interaction between host and isolate.

As shown in Table 4.1, cultivars could be ranked according to the extent of disease development in the order Doon Major (greatest),

TABLE 4.1: Disease assessment score (0-5) of three isolates of *E. cruciferarum* on leaf discs of four cruciferous hosts (120 hours after inoculation).

Isolate	Host				Mean
	Doon Major	Ruta Otofte	Barsica	RB25/8	
N2a	2.6	1.6	0.9	0.3	1.4
N2e	3.2	1.8	1.3	0.3	1.7
N3a	2.9	1.9	0.8	0.1	1.4
Mean	2.9	1.8	1.0	0.2	

SED ± (DF = 99): Host means = 0.20; Isolate means = 0.17; Host x Isolate = 0.34

Ruta Otofte, Barsica and RB25/8. Pathogen development was considerably greater on Doon Major, in the form of abundant mycelial growth, than on the other hosts: on Ruta Otofte there was moderate mycelial growth, while growth on Barsica was only sparse and on RB25/8 there was little development beyond the appressorial stage.

The incidence of fluorescent sites in relation to host tended to follow the same pattern as disease assessments, with Doon Major showing the greatest number and RB25/8 the least (Table 4.2). Isolate N2e produced significantly more fluorescent sites on Ruta Otofte and Barsica than other isolates.

TABLE 4.2: Numbers of fluorescent sites per colony produced by three isolates of *E. cruciferarum* on four cruciferous hosts (120 hours after inoculation).

Isolate	Host				Mean
	Doon Major	Ruta Otofte	Barsica	RB25/8	
N2a	13.9	1.5	0.3	0.3	4.0
N2e	14.3	3.4	2.3	0.3	5.1
N3a	15.2	1.4	0.5	0.3	4.3
Mean	14.5	2.1	1.1	0.3	

SED ± (DF = 33)
Host means = 0.55
Isolate means = 0.48
Host x Isolate = 0.95

TABLE 4.3: Number of necrotic cells per colony produced by three isolates of *E. cruciferarum* on four cruciferous hosts (120 hours after inoculation).

Isolate	Host				Mean
	Doon Major	Ruta Otofte	Barsica	RB25/8	
N2a	0.2	3.0	0.5	0.4	1.0
N2e	0.2	2.5	1.5	0.6	1.2
N3a	0.6	1.8	1.3	0.6	1.1
Mean	0.3	2.4	1.1	0.5	

SD ± (DF = 33)
Host means = 0.44
Isolate means = 0.38
Host x Isolate = 0.77

The lowest numbers of necrotic cells/colony were found with Doon Major and RB25/8 (Table 4.3). Numbers were intermediate with Barsica and highest on Ruta Otofte. No fluorescent sites or necrotic cells were found on the uninoculated controls.

Experiment 4.b: *Progress of colony development of E. cruciferarum*

(i) Fungal development

Germinated conidia of each isolate were found 4 hours after inoculation on all hosts. The germination rates of the two isolates were, in general, similar and at 12 hours 60% of conidia of both had, on average, germinated. The sequence of fungal development with time is illustrated in Figure 4.1. The percentage of infection units (conidia) at the germ tube stage was highest 12 hours after inoculation but was, on average, only 11%: as development progressed the percentage decreased until at 48 hours no conidia were found with germ tubes only. Germinated conidia with appressoria were observed firstly at 8 hours and, from then onwards, the counts of germinated conidia which formed appressoria were always greater than those for conidia with germ tubes only. At 12 hours, 39% of conidia were at the appressorium stage and by 24 hours the percentage was 80, but thereafter declined as fungal development progressed. However, even at 96 hours, an average of 30% of conidia remained at the appressorium stage. Primary hyphae, secondary hyphae and expanding colonies were first observed at 24, 48 and 72 hours respectively, with the percentages of conidia at each stage reaching a maximum 24 hours later. As with the previous two stages, after reaching a peak, a decline in the percentage of conidia at the primary

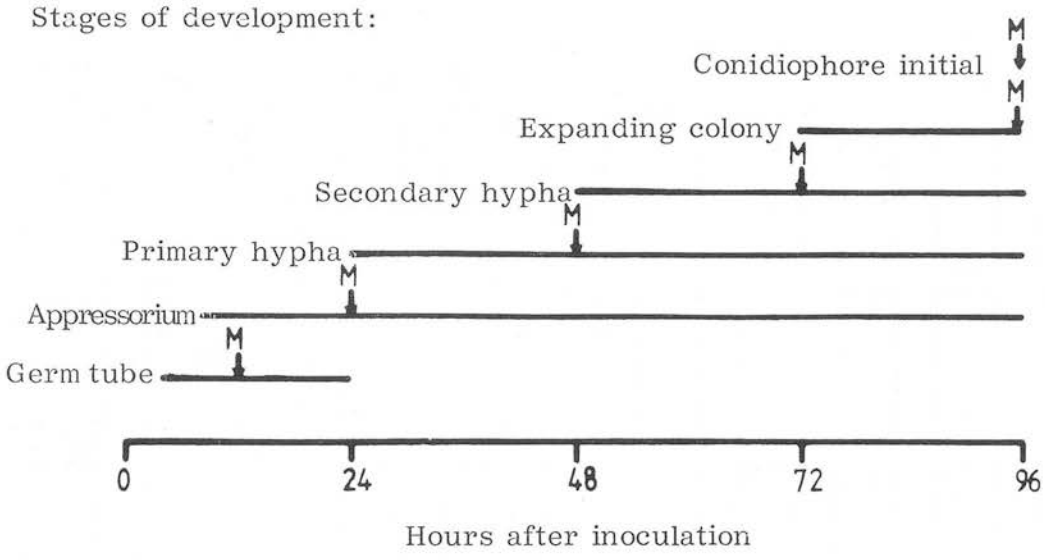


FIGURE 4.1: Periods over which different development stages were recorded and peak occurrence times (M).

hypha and secondary hypha stage was observed as colonies developed. Colonies with conidiophore initials were found 96 hours after inoculation when observations ceased.

From the analyses of variance of the data, the results of assessments for each stage of fungal development showed changes with time and significant main effects were associated with host and isolate, except for the germ tube stage: for all stages there were significant interactions between host and time after inoculation, between host, isolate and time and, except for the secondary hypha stage, between host and isolate. In Figure 4.2, which summarises the results of observations at each time of assessment, the variation in extent of fungal development in relation to time, host and isolate is illustrated: low numbers at any assessment time for any treatment relative to other treatments might reflect either a delay in conidia reaching a certain stage or a more rapid development to a subsequent stage.

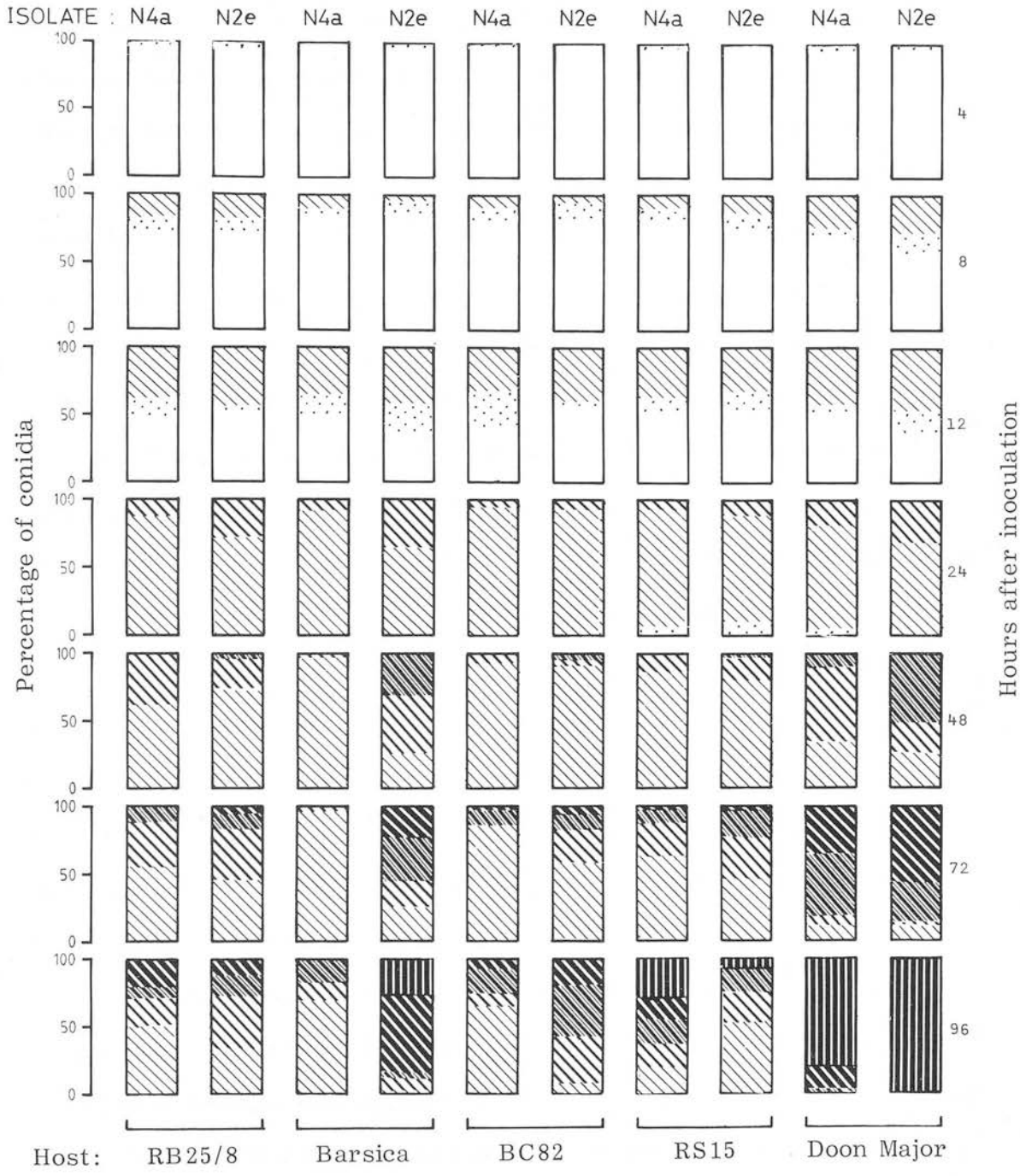


FIGURE 4.2: Percentage of infection sites of different stages of fungal development in relation to time, host and isolate of *E. cruciferarum*.

Key: Development stages

- ungerminated
- germ tube
- appressoria
- primary hypha
- secondary hypha
- expanding colony
- conidiophore initial
- conidia

The pattern of early development of each isolate on each of the hosts (Figure 4.3) indicates that germination and the production of appressoria were more or less similar for the two isolates on the five different hosts. Development beyond the appressorium stage was, however, clearly influenced by host factors. With Doon Major a more rapid development of primary hyphae, secondary hyphae, expanding colonies and conidiophore initials was evident compared with other hosts, which showed not only a slower development from stage to stage but a reduced percentage of infection sites which progressed to each subsequent stage. Among these other hosts, the degree of this retardation or inhibition of colony development varied and, development was notably limited for colonies on BC82, none of which had reached the conidiophore initial stage by 96 hours. Development of isolates on RB25/8 lagged behind that on RS15 and very few colonies on the latter host reached the conidiophore initial stage by 96 hours.

The interaction between host and isolate is also shown in Figure 4.3. On Barsica colony development of isolate N4a was inhibited while that of isolate N2e was relatively advanced, although significantly less than that of both isolates on Doon Major. A proportion of colonies of both isolates progressed to the conidiophore initial stage on RS15 but progress was slower with N2e than with N4a and, at 96 hours the percentages of colonies with conidiophore initials were 11 and 30 respectively. No consistent differences in the behaviour of the two isolates on RB25/8 were evident.

The extent of fungal development at 96 hours for each isolate/cultivar combination is illustrated in Figure 4.4. Doon Major was notable in that most conidia had produced sporulating colonies, although the numbers were less for isolate N4a which still showed nearly 14% of infection

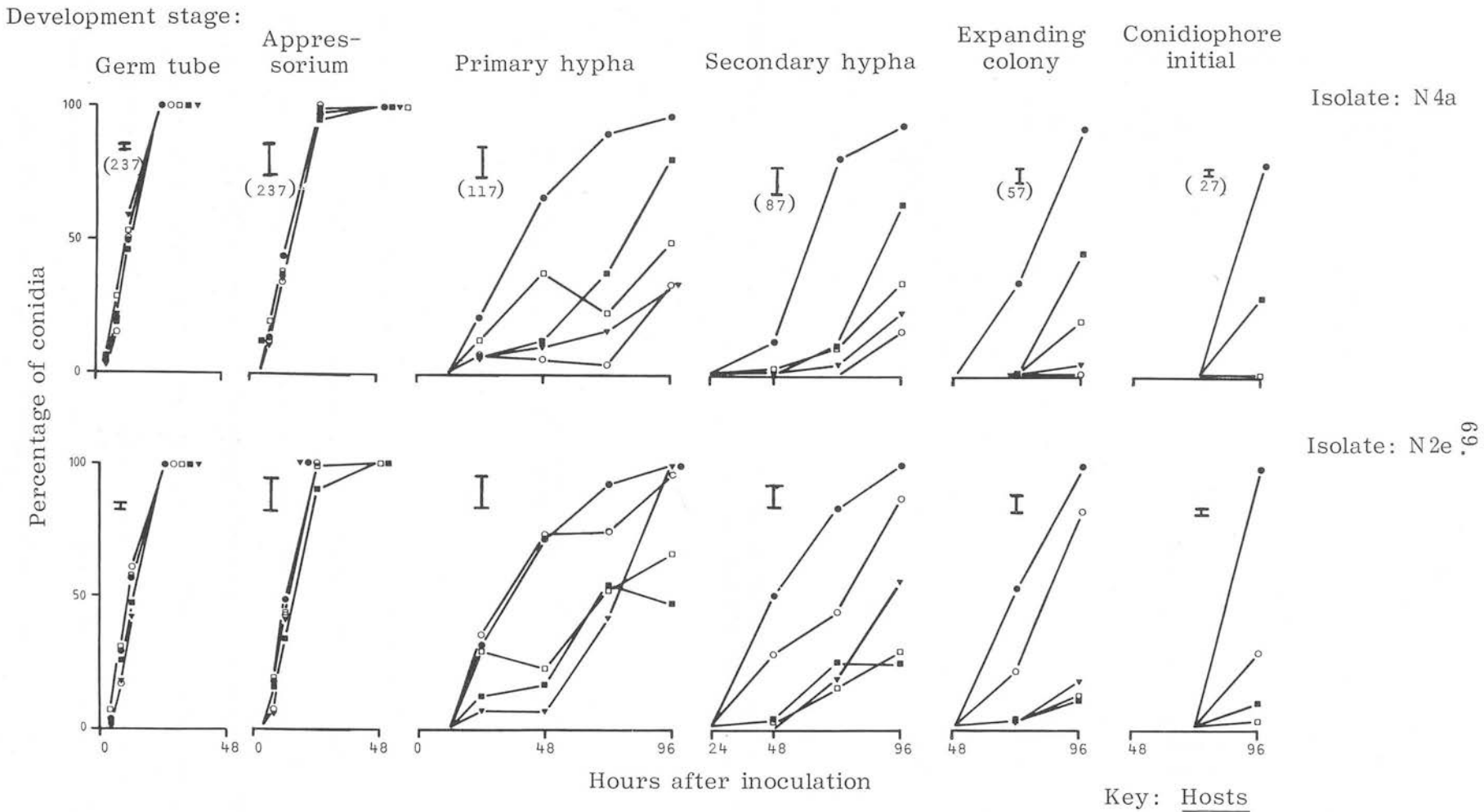


FIGURE 4.3: Percentage of conidia at or beyond each stage of development in relation to time for two isolates of *E. cruciferarum* on five hosts.

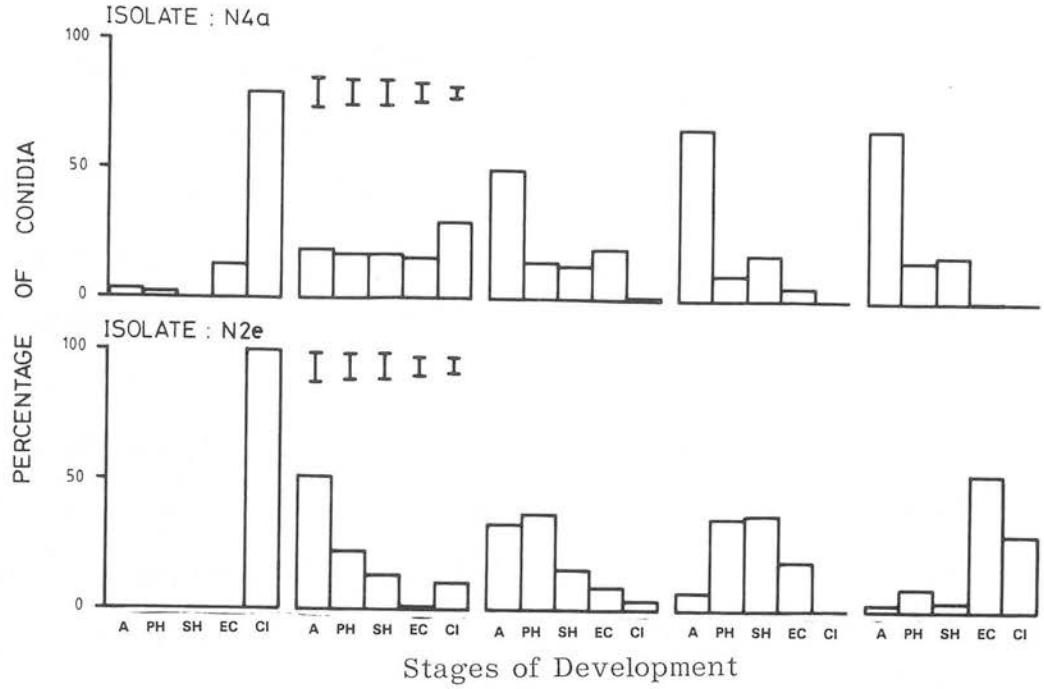


FIGURE 4.4: Percentage of infection sites at different stages of development at 96 hours after inoculation for two *E. cruciferarum* isolates on five hosts.

Key: Stages of development (DF)

A	Appressorium (237)	EC	Expanding colony (57)
PH	Primary hypha (117)	CI	Conidiophore initial (27)
SH	Secondary hypha (87)	I	SED

units at the expanding colony stage and 3% at the appressorium and at the primary hypha stages. In marked contrast to the development of isolates on Doon Major, no colonies reached the conidiophore initial stage on BC82, although the behaviour of the two isolates was different on this cultivar: the majority of N4a conidia (67%) failed to develop beyond the appressorium stage, while those of N2e had often developed primary hypha (36%) or secondary hypha (37%). Very few colonies of both isolates reached the conidiophore initial stage on RB25/8, although more conidia of N2e, compared with N4a, progressed beyond the appres-

sorium stage. In the case of Barsica and RS15 there were contrasting responses for the two isolates. With Barsica, N4a failed to produce sporulating colonies and few infection units reached the expanding colony stage, whereas approximately one-third of colonies of isolate N2e produced conidiophore initials and over half showed expanding colonies. On RS15 both isolates showed some development to the spore production stage: the proportion of sporulating colonies was greater with N4a, while a greater percentage of N2e conidia did not develop beyond the appressorial stage. With respect to isolate behaviour in general, N4a developed to the greatest extent on Doon Major and its development was less in decreasing order on RS15, RB25/8, BC82 and Barsica. Isolate N2e also developed best on Doon Major, but its development on other hosts ranked in the decreasing order of Barsica, BC82, RB25/8 and RS15: one-fifth of conidia reached the expanding colony stage on BC82 but no conidiophore initials were produced (Figure 4.4).

(ii) Fluorescent sites/colony

Fluorescent sites were found associated with conidia on Doon Major by 12 hours after inoculation but none were found on the other four hosts until 24 hours: no fluorescent sites were found at any time beneath conidia which had produced only a germ tube and none were found on any of the uninoculated controls. At 24 hours and later assessment times fluorescent sites were found associated with conidia which had produced an appressorium or developed further and there was an overall progressive increase in the number of fluorescent sites as colonies developed.

From the analyses of variance of the results for each stage of fungal development, from the appressorium stage to the expanding colony stage, the numbers of fluorescent sites/colony were found to vary significantly with host and, in most cases, with time after inoculation, but there were significant interactions between the factors. For colonies at the secondary hypha stage or at later stages of development, significant effects were also associated with isolate and its interaction with other factors.

At the appressorium and primary hypha stages of development, the mean number of fluorescent sites associated with each conidium was near one or less.

The number of fluorescent sites per colony at the secondary hypha stage ranged from 0 to 4.6 averaged for the different host/isolate/time combinations (Table 4.4). Doon Major tended to show the largest number at earlier times but, by 96 hours, numbers were greatest with RB25/8 or RS15 and intermediate with BC82 which showed slightly higher numbers than Barsica. In comparing isolates (Table 4.4), the number tended to be slightly more with N2e than with N4a but there was a significant interaction between host and isolate: N2e showing significantly higher numbers than N4a on Barsica at 48 and 72 hours. In contrast, with BC82 isolate N4a tended to show larger numbers of fluorescent sites although the differences were not significant. Fluorescent site numbers were higher with N2e than with N4a on cultivars Doon Major at 72 hours, and on RB25/8 at 96 hours.

With expanding colonies, numbers of fluorescent sites ranged from 0 to 7.7 for different host/isolate combinations (Table 4.4). Numbers

TABLE 4.4: Number of fluorescent sites associated with colonies at different stages of development showing the interaction between host, isolate and time of observation.

Development stage	Hours after inoculation	Host: RB25/8 Isolate: N4a	RB25/8 N2e	Barsica N4a	Barsica N2e	BC82 N4a	BC82 N2e	RS15 N4a	RS15 N2e	Doon Major N4a	Doon Major N2e	SED ±
Secondary hypha	48	0.5	0.9	0	2.2	0.3	0	0	0.5	1.5	1.4	0.80 (DF = 87)
	72	3.2	2.5	0	4.6	2.6	1.8	2.4	2.8	2.6	4.1	
	96	2.4	4.5	1.7	1.6	3.2	1.8	3.1	3.0	0.1	-	
Expanding colony	72	1.0	4.1	0	5.2	1.4	1.6	0.8	5.3	5.8	6.6	5.62 (DF = 57)
	96	6.8	7.0	0	7.7	2.5	4.7	6.9	7.1	6.3	-	
Conidiophore initial	96	7.0	10.5	-	9.7	-	-	9.8	8.2	12.2	13.6	2.86 (DF = 27)

were usually higher on Doon Major, RB25/8 and RS15 than on other hosts, but there were significant interactions between isolate and host and isolate, host and time. With Barsica, fluorescent sites were observed only with isolate N2e and N2e showed greater numbers than N4a at 72 hours on RB25/8 and RS15.

For colonies with conidiophore initials, seen at 96 hours, the frequency of fluorescent sites ranged from 7 to 13.6 for various host/isolate combinations (Table 4.4). No colonies in this category were observed on BC82 or on Barsica with isolate N4a. Numbers of fluorescent sites were higher on Doon Major than on other hosts producing colonies with conidiophore initials.

The numbers of fluorescent sites which developed beneath infection units, irrespective of their stage of development, were similar for both isolates on all hosts at 24 and 48 hours after inoculation (Figure 4.5). However, at 72 and 96 hours substantial differences were found not only between hosts but also between isolates on the same host. The greatest numbers of fluorescent sites were found beneath colonies of both isolates, particularly isolate N2e, on Doon Major. On the four remaining hosts the number of fluorescent sites beneath colonies of isolate N2e were intermediate on Barsica and low on RS15, RB25/8 and BC82: on the other hand, beneath colonies of isolate N4a, numbers were lowest on Barsica, also low on BC82, and intermediate on RS15 and RB25/8.

(iii) Necrotic host cells/colony

No necrotic cells were found on uninoculated leaf discs, but necrotic epidermal cells were recorded 24 hours after inoculation on all cultivars except Doon Major. The average numbers of necrotic cells generally

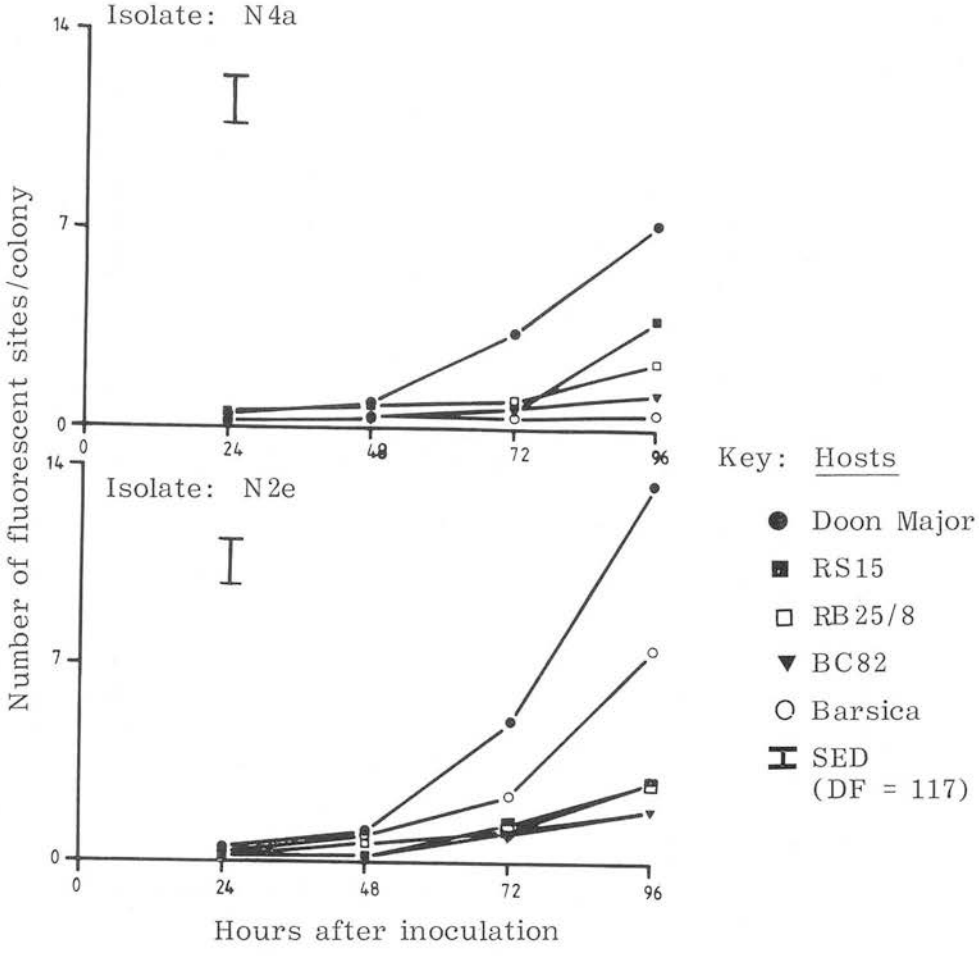


FIGURE 4.5: Number of fluorescent sites at each assessment time on five hosts inoculated with two isolates of *E. cruciferarum*. (Mean of 50 colonies.)

increased at subsequent times of observation: with Doon Major, necrotic cells were first observed at 48 hours but numbers remained very small. Significant differences in the numbers of necrotic cells were associated with different hosts but the main effects of isolate were not significant. However, significant interactions were found between host and time and host and isolate. The host/isolate interaction was consistent over time.

As already noted, Doon Major produced few or no necrotic cells in response to infection, whereas at the appressorium, primary hypha

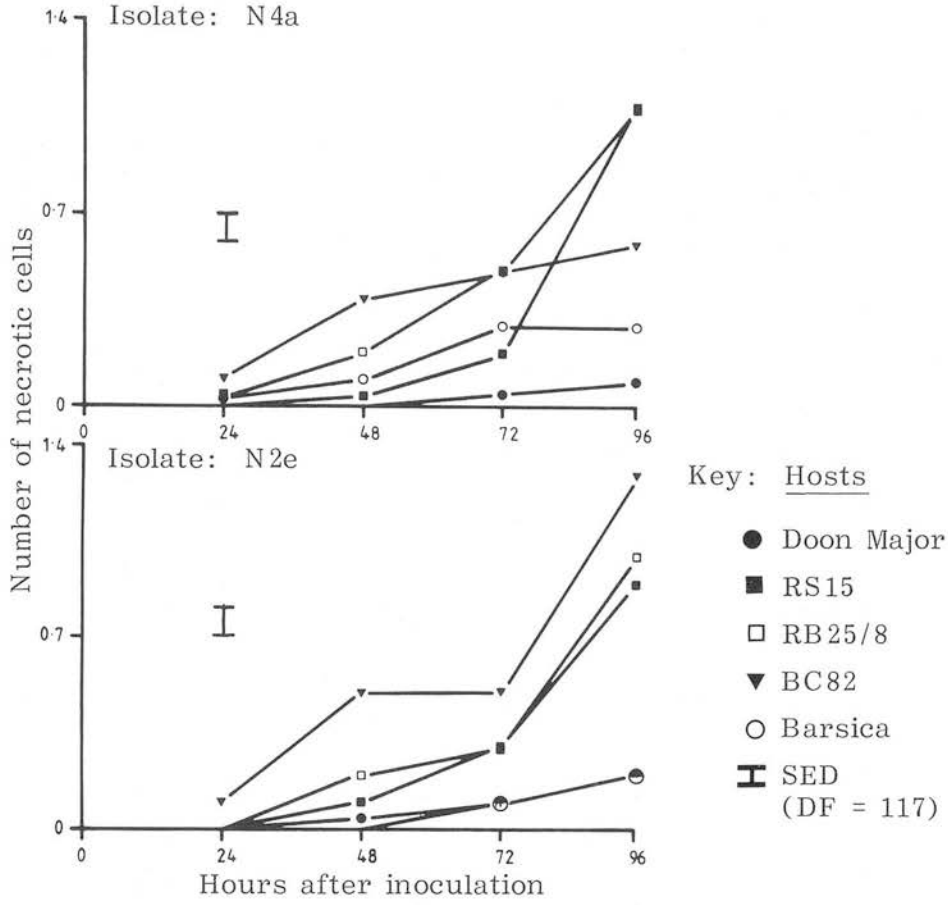


FIGURE 4.6: Number of necrotic cells at each assessment time on five hosts inoculated with two isolates of *E. cruciferarum*. (Mean of 50 colonies.)

and secondary hypha stages the average numbers/colony of necrotic cells were always relatively high with BC82. At later times of assessment and with later stages of development, the incidence of necrosis tended to become high with RB25/8 and RS15. With Barsica numbers of necrotic cells were very low, particularly where isolate N2e was used as inoculum (Table 4.5). There was, however, a tendency for N2e to give more necrosis on BC82 than isolate N4a.

With isolate N2e, necrotic cells developed earlier and were always more frequent on BC82, while on leaf discs of Doon Major and Barsica their occurrence was delayed and limited (Figure 4.6). Hosts RB25/8

TABLE 4.5: Number of necrotic cells associated with colonies at different stages of development showing the interaction between host and isolate.

Development stage	Host: RB25/8		Barsica		BC82		RS15		Doon Major		SED ±
	Isolate: N4a	N2e	N4a	N2e	N4a	N2e	N4a	N2e	N4a	N2e	
Appressorium	0.2	0.2	0.2	0	0.3	0.6	0.2	0.3	0.1	0.1	0.04 (DF = 117)
Primary hypha	0.4	0.4	0.2	0	0.3	0.5	0.4	0.3	0	0	0.07 (DF = 117)
Secondary hypha	1.3	0.7	0.2	0.1	0.9	1.2	0.8	1.1	0.1	0	0.12 (DF = 87)
Expanding colony	2.4	2.0	0	0.1	0.3	0.7	2.4	0.6	0.3	0.2	0.91 (DF = 57)
Conidiophore initial	2.0	1.8	-	0.3	-	-	1.3	2.7	0.1	0.2	0.89 (DF = 27)

and RS15 occupied an intermediate position. With isolate N4a, there was again a more frequent occurrence of necrosis at earlier times of assessment on BC82, but at 96 hours RB25/8 and RS15 showed a greater response. At this time, Doon Major and Barsica again showed a low incidence of necrosis, particularly Doon Major.

Experiment 4.c: *Progress and extent of colony development*

(i) Fungal development

Germinated conidia of each isolate on all cultivars were found 4 hours after inoculation when the maximum proportion in this category (8%) occurred (Figure 4.7). The percentage decreased to 1% at 18 hours as fungal development progressed, and no conidia with germ tubes only were found at later times. Germinated conidia with appressoria were also found at 4 hours, and increased in numbers to show a maximum of 87%, on average, at 18 hours: subsequently percentages declined but even at the last assessment, 144 hours after inoculation, an average of almost 14% of conidia had not developed further. Primary hyphae were first observed at 18 hours and the percentage at this stage reached a maximum at 48 hours of 45%: thus, while maximum numbers of conidia at the appressorium stage were evident 14 hours after they were seen firstly, the percentage with primary hyphae reached a peak 30 hours after their first occurrence. Conidia with secondary hyphae and conidia producing expanding colonies were observed firstly at 48 and 72 hours respectively, and the percentage at the two stages were found to be highest (28% and 14%) 24 hours later. Sporulating colonies were recorded at 144 hours after inoculation: at this time an average percentage of 7% and 1% of conidia were found at the conidiophore initial or conidial stage respectively.

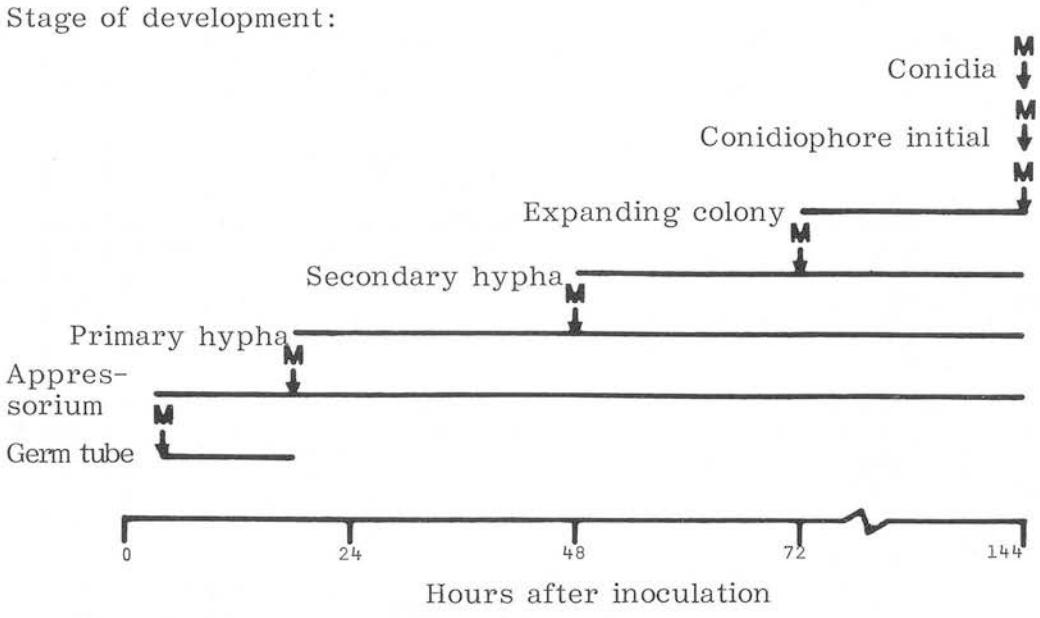


FIGURE 4.7: Periods over which different development stages were recorded and peak occurrence times (M).

The numbers of conidia with germ tubes at the earlier times of assessment differed significantly for the different isolates, but there were no significant differences between hosts and no significant interaction between the host and isolate: isolate O4a tended to show higher numbers and N4a fewer (Figure 4.8). For all other development stages, significant differences were found for isolate and host effects as well as time; significant interactions between the factors also occurred. Figure 4.8 shows the results of counts of conidia in each development stage for different host/isolate combinations of each assessment time, illustrating the variation associated with different experimental factors: as noted in the previous experiments, low numbers at a particular development stage at any assessment time for any treatment, relative to other treatments, may reflect delay in reaching a certain stage or a more rapid development to a subsequent stage.

From Figure 4.9 and Table 4.6, the pattern and rate of development of each isolate on the four hosts can be seen. Spore germination

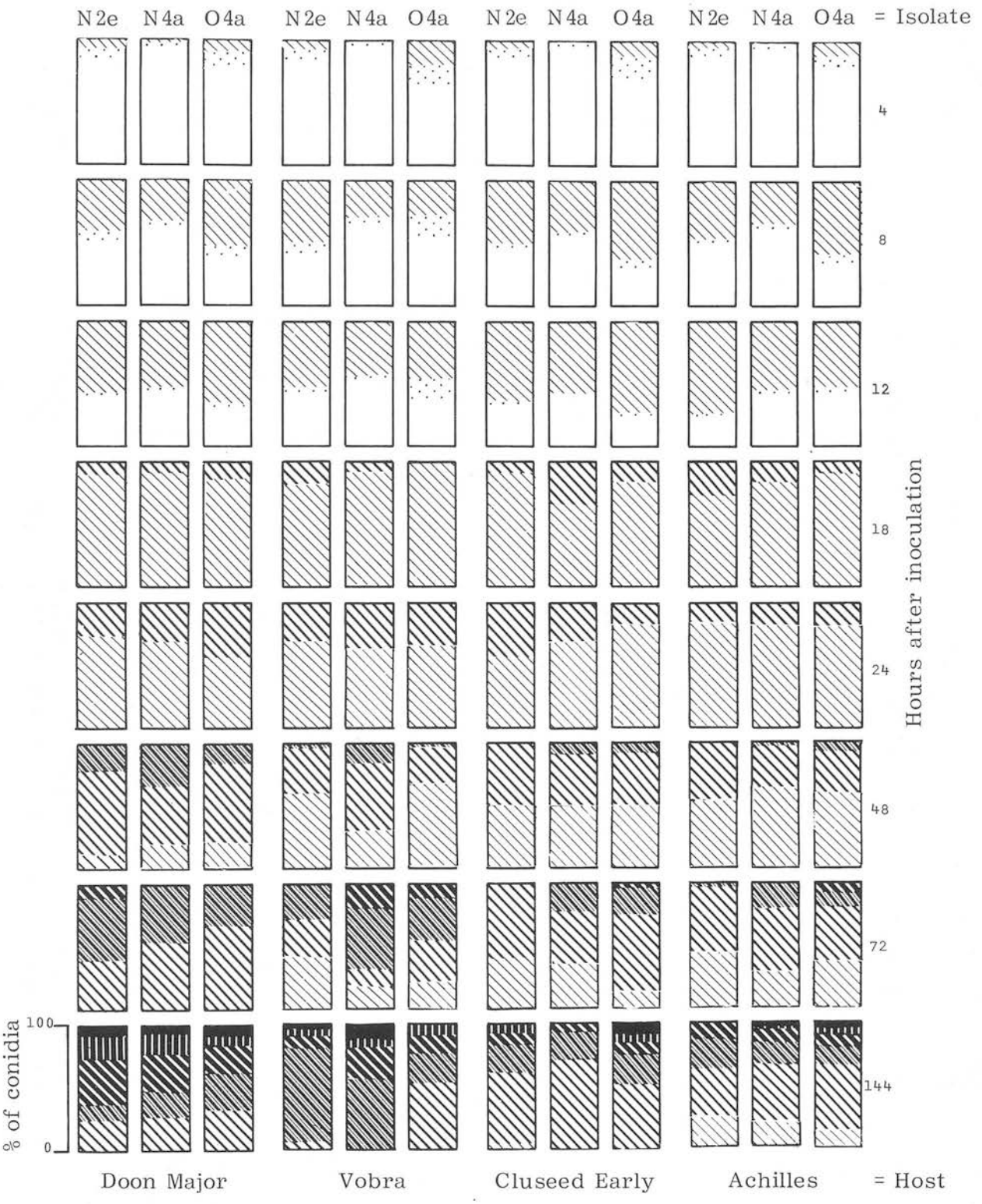


FIGURE 4.8: Percentage of infection sites at different stages of fungal development in relation to time, host and isolate of *E. cruciferarum*.

Key: see Figure 4.2.

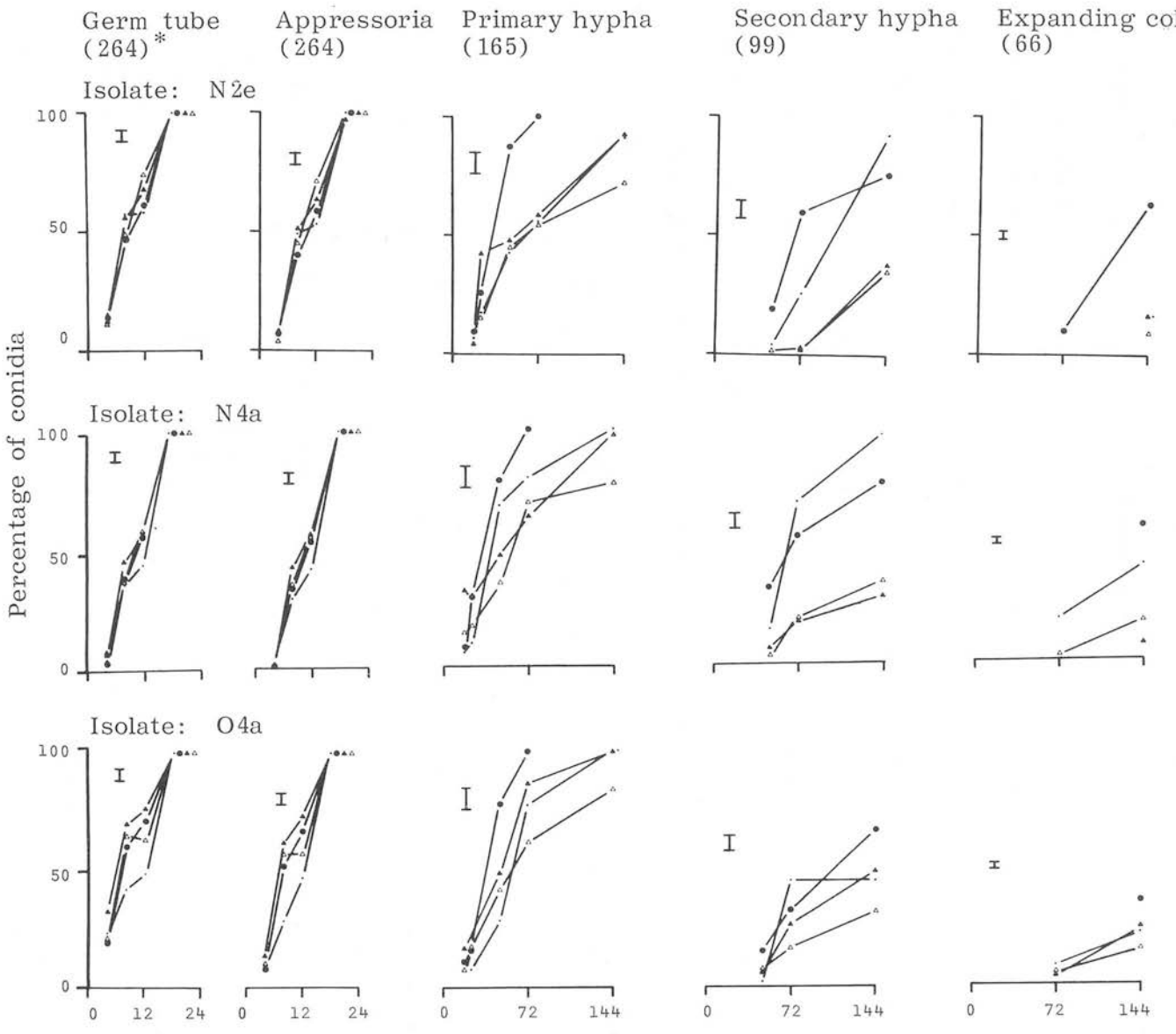
FIGURE 4.9:

Percentage of conidia at or beyond each stage of development in relation to time for three isolates of *E. cruciferarum* on four hosts.

Key: Hosts

- ● Doon Major
- · Vobra
- ▲ Cluseed Early
- △ Achilles

( )* DF



and development of appressoria of each isolate were generally completed within 18 hours after inoculation. Germination was slower during the first 12 hours on Vobra than on other hosts, especially with isolate O4a, but at 18 hours no differences were observed between hosts or isolates. With respect to the effect of host on the rate and total percentage of conidia which developed to the primary hypha, secondary hypha, expanding colony, conidiophore initial and conidia stages, all isolates on Doon Major developed to each stage, with few exceptions, more rapidly and, also, more frequently to advanced stages than they did on either Vobra, Cluseed Early or Achilles. However, isolates

TABLE 4.6: Percentage of infection sites at sporulation stages 144 hours after inoculation for three isolates of *E. cruciferarum* on four hosts.

	Conidiophore initial stage or beyond				Conidia production stage			
Host	N2e	N4a	O4a	Mean	N2e	N4a	O4a	Mean
Doon Major	25.5	25.0	12.5	21.0	7.5	7	4.5	6.3
Vobra	4.0	18.5	6.0	9.5	0	10	0	3.3
Cluseed Early	8.0	0.5	16.0	8.2	0	0	8	2.7
Achilles	2.0	4.0	6.0	4.0	0	0	2.5	0.8
Mean	9.9	12.1	10.1		1.9	4.3	3.7	
	SED ± (DF = 42)				SED ± (DF = 42)			
Host	0.18				0.10			
Isolate	0.16				0.08			
Host x Isolate	0.08				0.48			

behaved differently on different hosts: the extent of colony development on Doon Major was less advanced with isolate O4a compared with other isolates. Isolate N4a was found to develop more rapidly and to a greater extent than isolates N2e or O4a on Vobra. On the other hand, isolate O4a generally developed more rapidly and to a more advanced stage on Cluseed Early than isolates N2e or N4a: O4a was the only isolate to reach the conidial stage on Cluseed Early and Achilles. Moreover, unlike the other two isolates, O4a produced more colonies at advanced stages of development on Cluseed Early than on Doon Major.

Adverse host effects on the development of fungal isolates were reflected in a reduced percentage of colonies which reached more advanced stages and, also, in a delay in the transition from one stage to the next: thus, whereas isolates N4a and O4a had produced secondary hyphae on Cluseed Early 48 hours after inoculation, colonies of N2e at this stage were first observed only at 72 hours. At 144 hours all host/isolate combinations showed some colonies with conidiophore initials, but the frequency varied and delayed conidial production was found with isolate N2e and O4a on Vobra, and isolates N2e or N4a on Cluseed Early or Achilles.

From Figure 4.10 it is seen that colony development culminating in conidiophore development and conidial production at 144 hours occurred most consistently on Doon Major: on this cultivar all germinated conidia developed beyond the appressorium stage. In contrast, a proportion of conidia of two isolates failed to develop beyond the appressorium stage on Vobra, 3% and 7% with isolates N4a and N2e respectively. On Achilles 15 to 25% of germinated conidia did not develop beyond the appressorium stage and a substantial proportion showed no development beyond the primary hypha category. On Cluseed Early isolates behaved

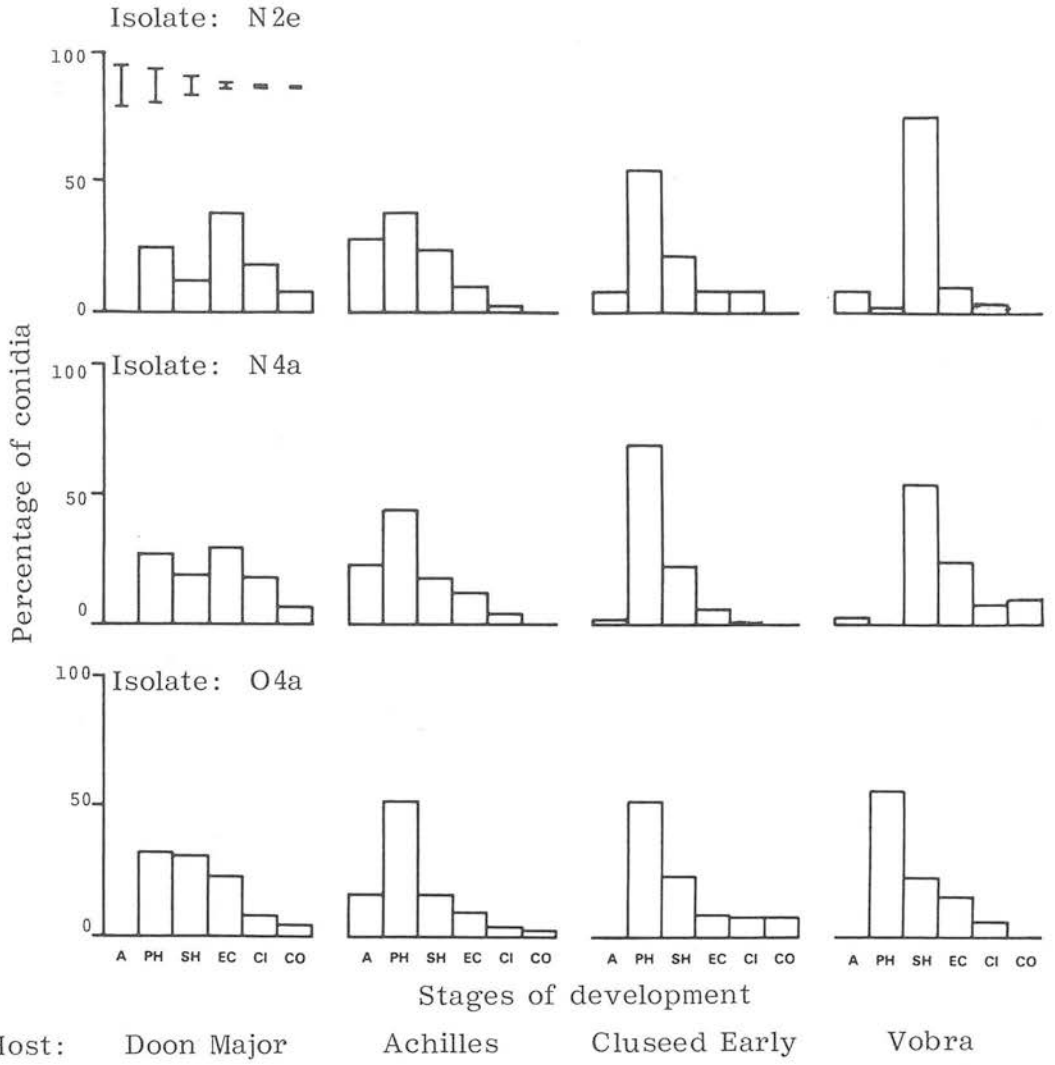


FIGURE 4.10: Percentage of infection sites at different stages of development at 144 hours after inoculation for three *E. cruciferarum* isolates on four *Brassica* hosts.

Key: Stages of development (DF)

A	Appressorium (264)	EC	Expanding colony (66)
PH	Primary hypha (165)	CI	Conidiophore initial (33)
SH	Secondary hypha (99)	CO	Conidia produced (33)

differently: a small proportion of infection units of isolates N2e and N4a with appressoria failed to grow further but their development mainly stopped at the primary hypha stage: infection units of isolate O4a all developed beyond the appressorium stage and although a substantial

proportion stopped at the primary or secondary hypha stage, about 25% reached the expanding colony stage or beyond.

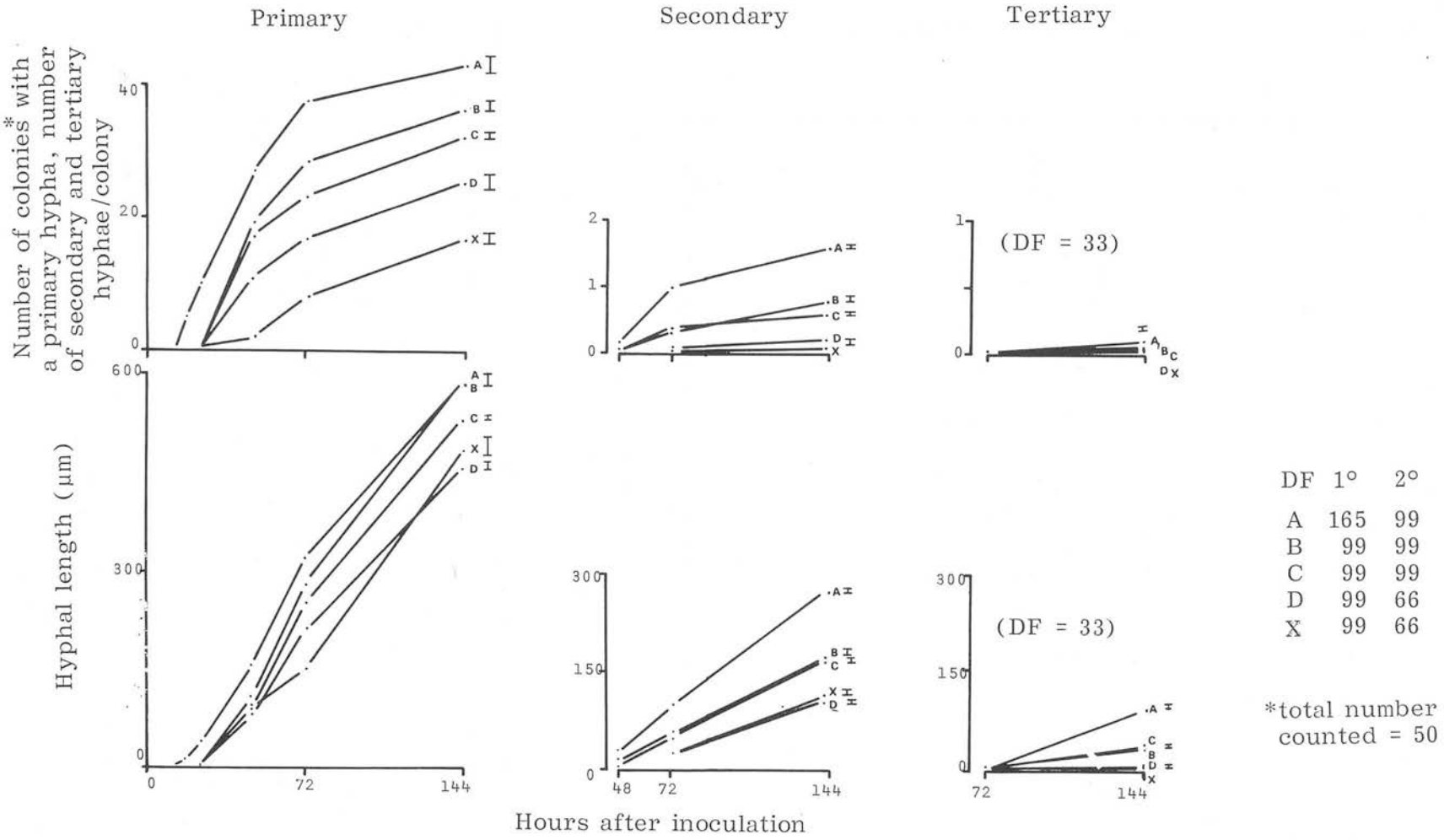
(ii) Hyphal development

On all hosts primary, secondary and tertiary hyphae were found by 18, 48 and 144 hours after inoculation respectively and, with the exception of tertiary hyphae, increased in number and length with time after inoculation. The development of primary, secondary and tertiary hyphae with time are illustrated in Figures 4.11 to 4.15.

The time of initiation, number and length of hyphae were influenced by position. Hyphae at position A were always most numerous, branched most and were generally the longest: however, primary hyphae at position B achieved similar lengths by 144 hours. The number and length of primary C hyphae were intermediate but their branching was similar to that of B hyphae. D and X hyphae showed the least increase in numbers, branching and length (Figure 4.11).

The development of hyphae at each position varied significantly with host and, with few exceptions, isolate: significant interactions were found, however, between host, isolate and time. Greater numbers and longer hyphae were produced on Doon Major than on any other host: numbers of primary hyphae of Vobra occupied an intermediate position above that of the other two hosts but secondary hyphal development on Vobra tended to be less than that of Cluseed Early and Achilles (Figure 4.12).

Each isolate produced its largest number of hyphae on Doon Major: on this cultivar and Vobra more hyphae were produced by colonies isolate N4a, in comparison with other isolates. No significant differences in numbers were found between colonies of the three isolates on Achilles,



DF	1°	2°
A	165	99
B	99	99
C	99	99
D	99	66
X	99	66

*total number counted = 50

FIGURE 4.11: General pattern of primary, secondary and tertiary hyphal development.

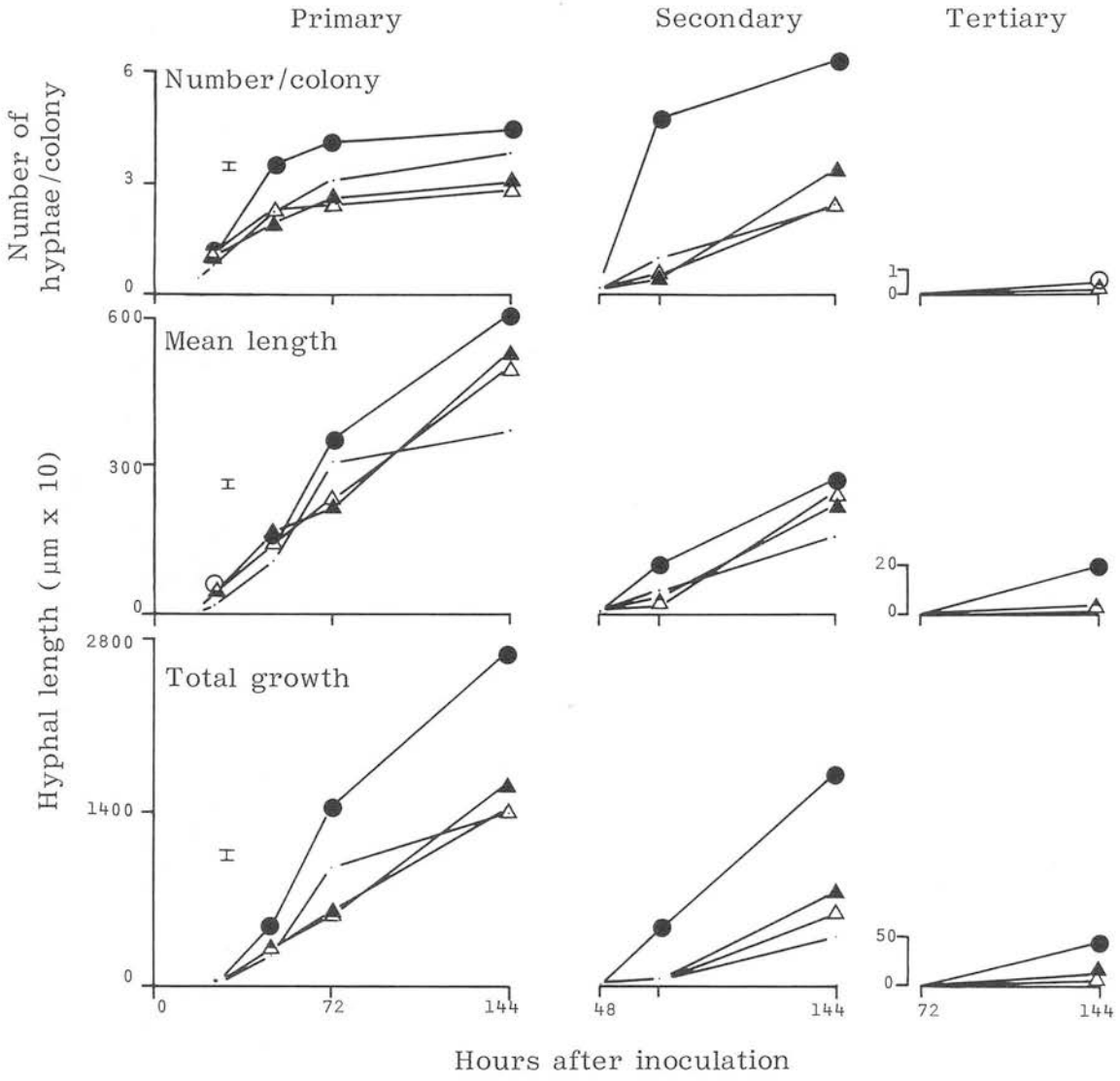


FIGURE 4.12: Extent of primary, secondary and tertiary hyphal development of *E. cruciferarum* isolates on four *Brassica* hosts.

Key: Host

- ● Doon Major
- · Vobra
- ▲ Cluseed Early
- △ Achilles

I SED (DF = 165)

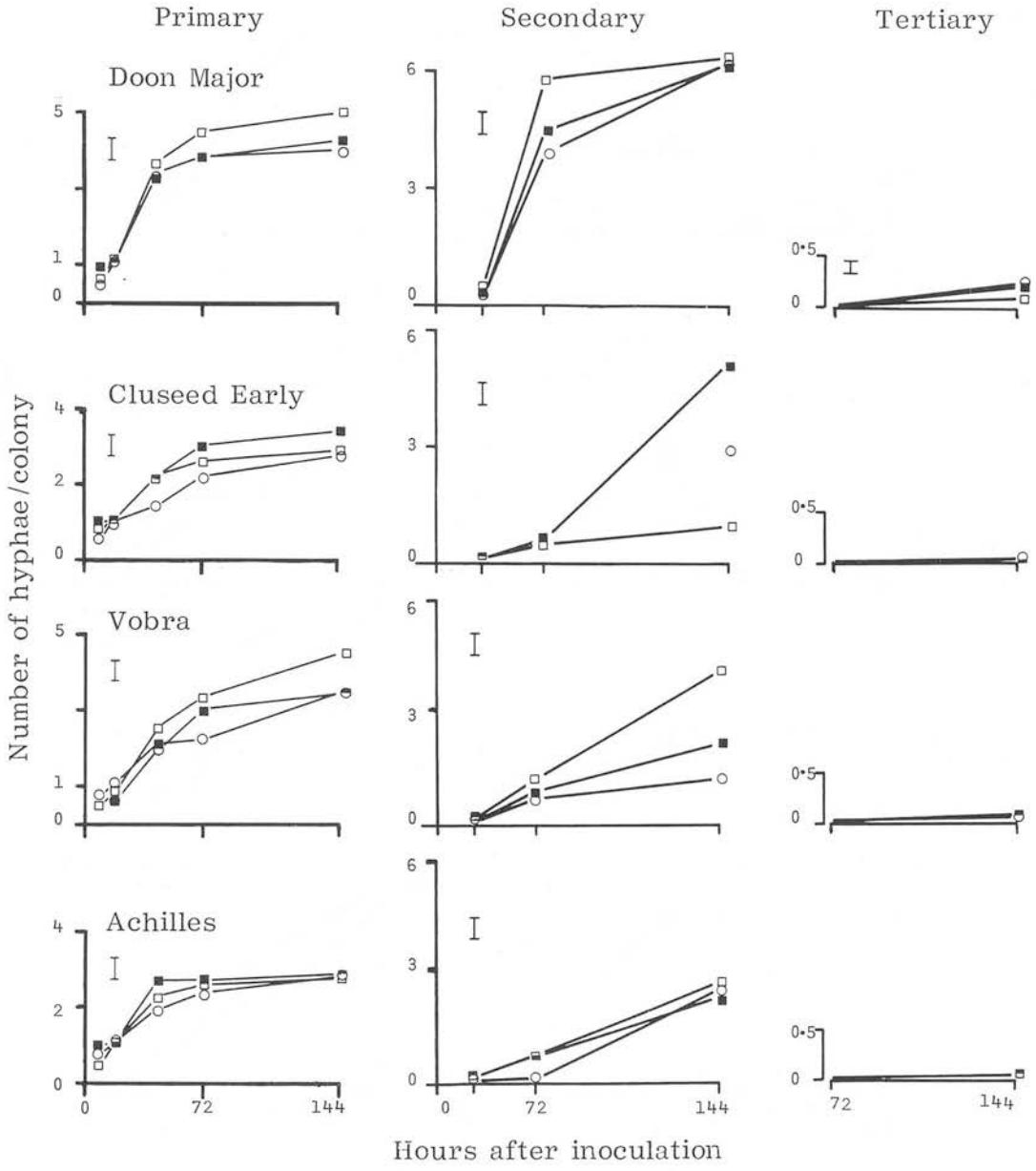


FIGURE 4.13: Number of primary, secondary and tertiary hyphae per colony of three *E. cruciferarum* isolates on four *Brassica* plants.

Key: Isolates

- ■ O4a
- □ N4a
- ○ N2e
- I SED (DF = 165)

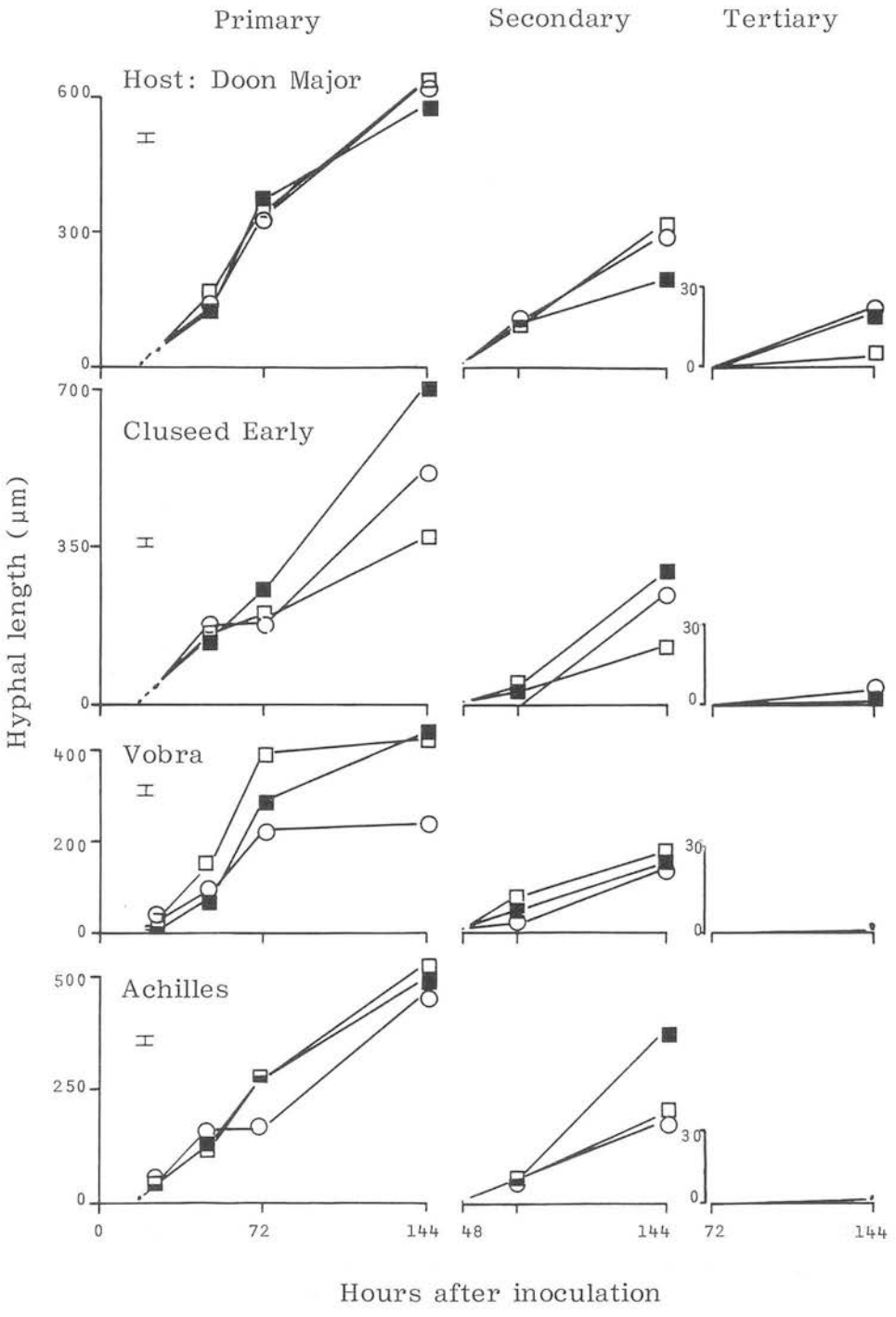


FIGURE 4.14: Mean length of primary, secondary and tertiary hyphae per colony of three *E. cruciferarum* isolates on four *Brassica* hosts.

Key: Isolate

- □ N4a
- ■ O4a
- ○ N2e
- I SED (DF = 165)

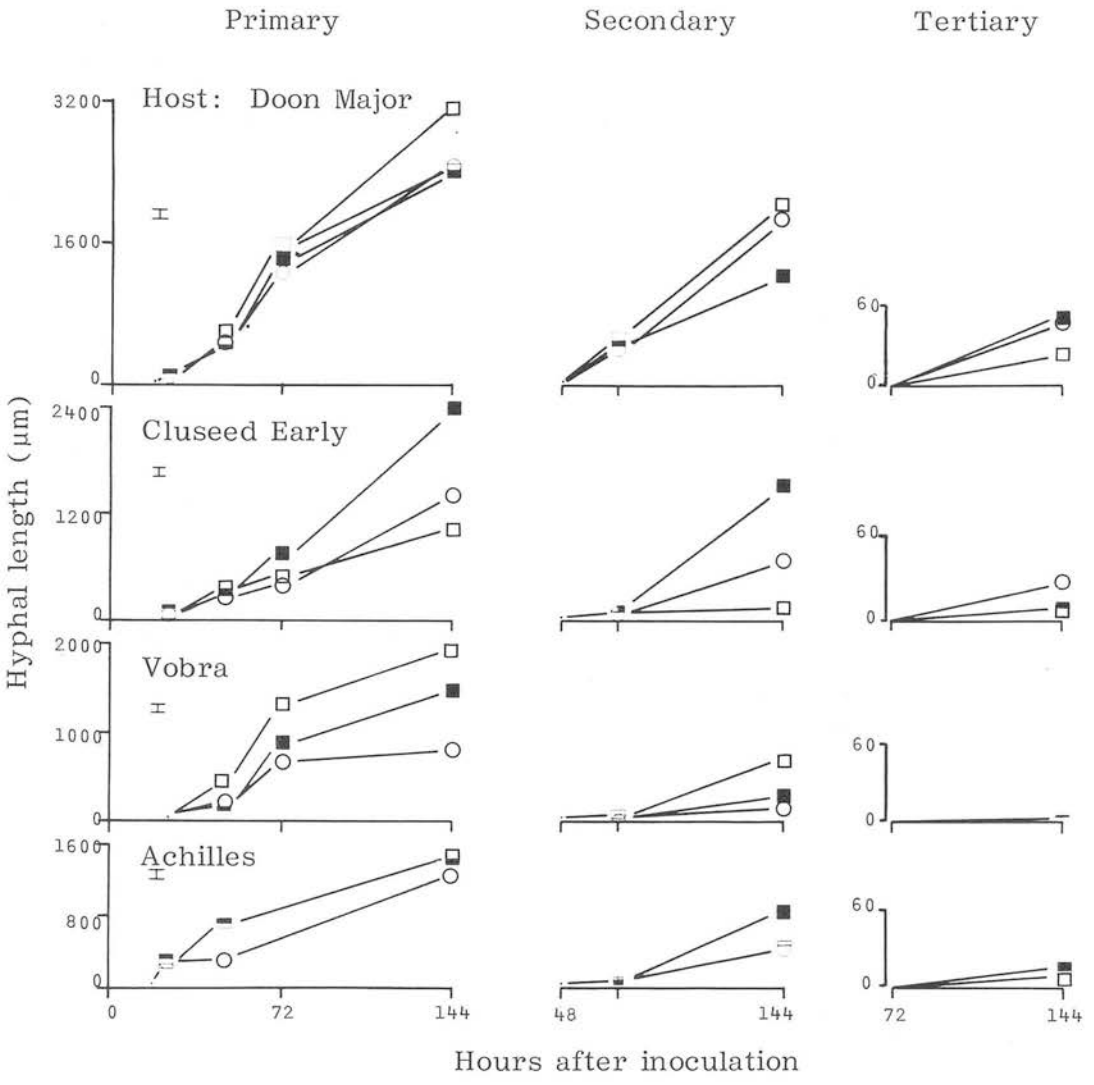


FIGURE 4.15: Total primary, secondary and tertiary hyphal growth (number x length) per colony of three *E. cruciferarum* isolates on four *Brassica* hosts.

Key: Isolate

□ N4a

■ O4a

○ N2e

I SED (DF = 165)

but isolate O4a tended to produce most on Cluseed Early (Figure 4.13). The mean lengths and total hyphal growth of isolates were greatest on Doon Major, except in the case of O4a which developed to a greater extent on Cluseed Early (Figures 4.14 and 4.15). The mean hyphal lengths of different isolates on Doon Major were similar, but isolate N4a gave the greatest total growth. On Vobra and Achilles, longer hyphae and a greater total growth were produced by isolates N4a and O4a than by N2e: on Cluseed Early hyphal length and total growth were greatest with O4a, intermediate with N2e and least with N4a colonies.

(ii) Conidiophore production

Sporulation from colonies was seen only at the assessments made 144 hours after inoculation, when conidiophores were found on all cultivars. The average number of conidiophores produced from primary hyphae was influenced by hyphal position, and varied significantly with cultivar and isolate and their interaction. The average number of conidiophores was greatest from hyphae at position A and decreased in order B to X (Table 4.7). Colonies on Doon Major and Cluseed Early produced more conidiophores than colonies on Achilles, and very few were formed on average on Vobra.

Isolate O4a on average produced the largest number of conidiophores and N4a the least, but the behaviour of each of the three isolates on different hosts varied considerably (Figure 4.16). Isolate N2e produced its highest numbers of conidiophores on Doon Major, whereas isolate O4a and N4a produced their highest numbers on Cluseed Early and Achilles respectively: conidiophore frequency of isolate O4a on Cluseed Early was greater than those found with any other host/isolate combination. Conidiophores on Doon Major were most numerous with colonies

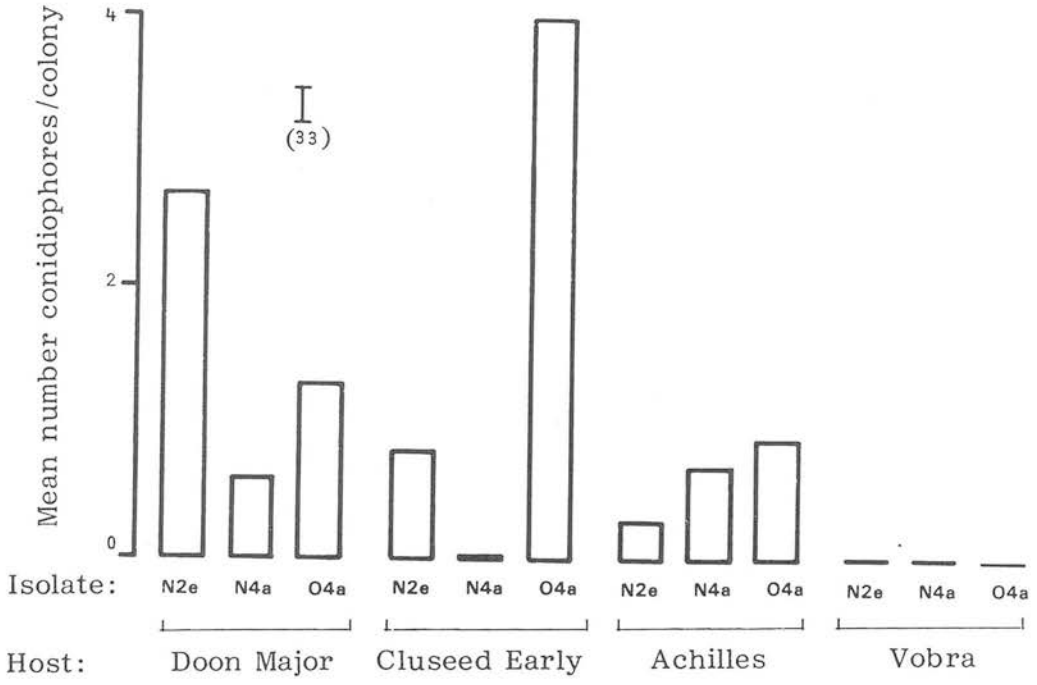


FIGURE 4.16: Number of conidiophores/colony* of three isolates of *E. cruciferarum* on four *Brassica* hosts.

*(A + B + C + D + X)

TABLE 4.7: Number of conidiophores produced by hyphae from each primary hyphal position by *E. cruciferarum* isolates on four *Brassica* hosts.

Hyphal position	Cultivar				Cultivar SED (DF = 33)
	Doon Major	Cluseed Early	Achilles	Vobra	
A	0.6	0.6	0.3	<0.1	0.04
B	0.4	0.3	0.1	<0.1	0.03
C	0.2	0.2	0.1	<0.1	0.01
D	0.2	0.2	0.1	0	0.01
X	0.1	0.1	<0.1	0	0.01

of N2e, intermediate with O4a and least with isolate N4a: on Cluseed Early colonies of O4a gave the largest numbers with N2e intermediate, while conidiophore production was negligible with N4a colonies. On Achilles conidiophore development was moderate or poor: numbers were highest with O4a and least with N2e colonies. All three isolates showed only very little or no conidiophore production on Vobra.

(iv) Fluorescent sites

Large and small fluorescent sites were found 18 hours after inoculation in association with colonies which had reached or developed beyond the primary hyphal stage of development and numbers increased with time after inoculation: no fluorescent sites were found on any of the uninoculated controls.

Both large and small fluorescent sites were recorded firstly beneath primary A hyphae, at 18 hours, beneath primary B and C hyphae at 48 hours, then with D and X hyphae at 72 hours (Figure 4.17). At each assessment time from 18 to 72 hours, greater numbers of fluores-

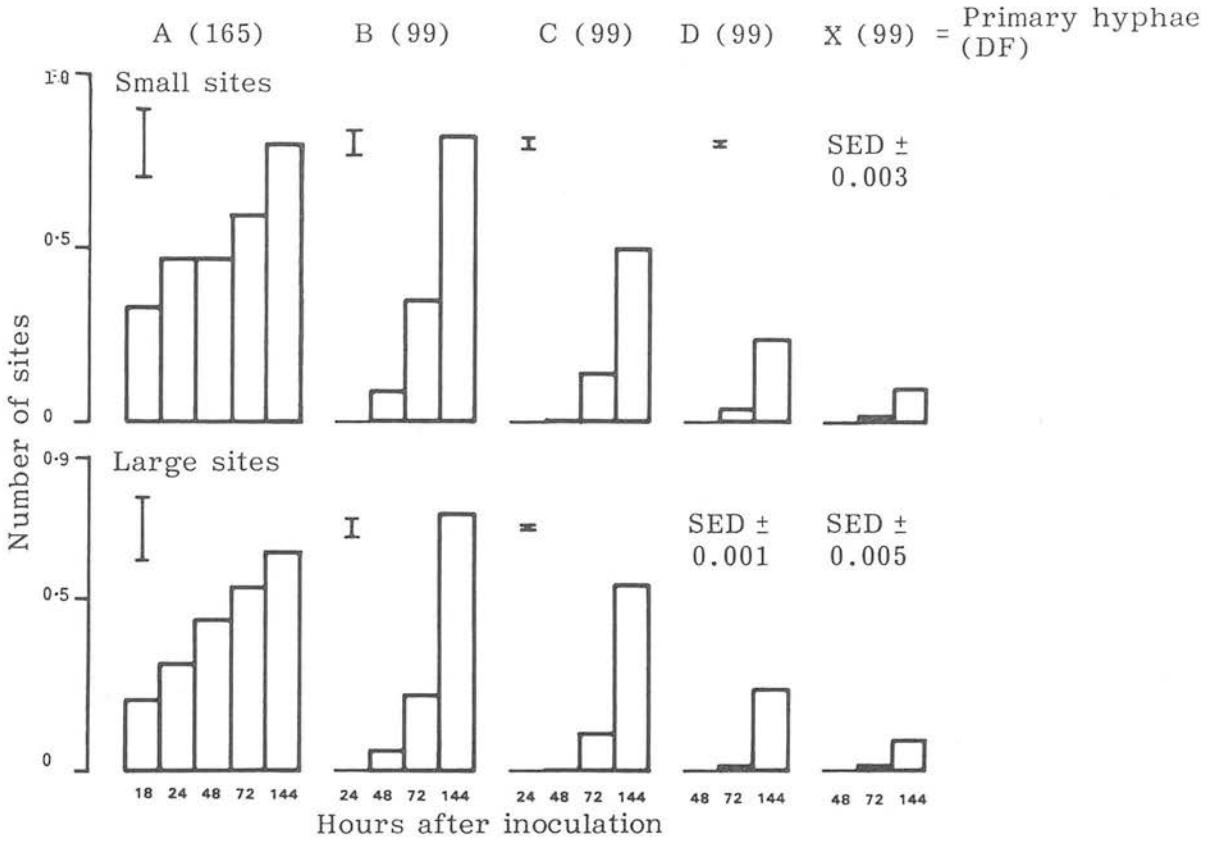


FIGURE 4.17: Number of small (<7 µm) and large (>7 µm) fluorescent sites associated with primary hyphae (A, B, C, D, X). (Mean of all isolates and hosts.)

cent sites were found with primary A hyphae and in decreasing order of primary B, C, D and X hyphae. However, at 144 hours after inoculation numbers were similar with A and B positions.

The numbers of large and small fluorescent sites found with each hypha tended to follow the same pattern of production as illustrated in Figure 4.17 on each of the four hosts. However, numbers of fluorescent sites/colony, i.e. total number beneath primary A, B, C, D and X hyphae, differed between hosts. At each assessment time up to 72 hours after inoculation, differences in the number of small sites were generally slight, but at 144 hours substantially more were found beneath colonies on Doon Major than on other hosts (Figure 4.18a). The numbers of large fluorescent sites/colony (Figure 4.18b) were generally greater with Doon Major, than with any of the other three hosts at all times. From Figure 4.18 it is seen that, throughout the period of assessment, with the hosts Cluseed Early, Vobra and Achilles numbers of small fluorescent sites/colony exceeded the number of large sites/colony. With Doon Major the reverse applied.

The variation in the total number of fluorescent sites (large and small)/colony between isolates on each of the four hosts is summarised in Figure 4.19. On Doon Major the range of variation was widest, N4a showed the greatest number and O4a the least. On Vobra, isolate N4a and also O4a gave greater numbers than N2e. On Cluseed Early O4a gave significantly more fluorescent sites than N2e and N4a. The ranking of isolates was the same on Achilles as on Cluseed Early but the differences were small. With the hosts Doon Major and Cluseed Early, on which isolates exhibited the greatest variation, both small and large sites showed more or less the same relative positions.

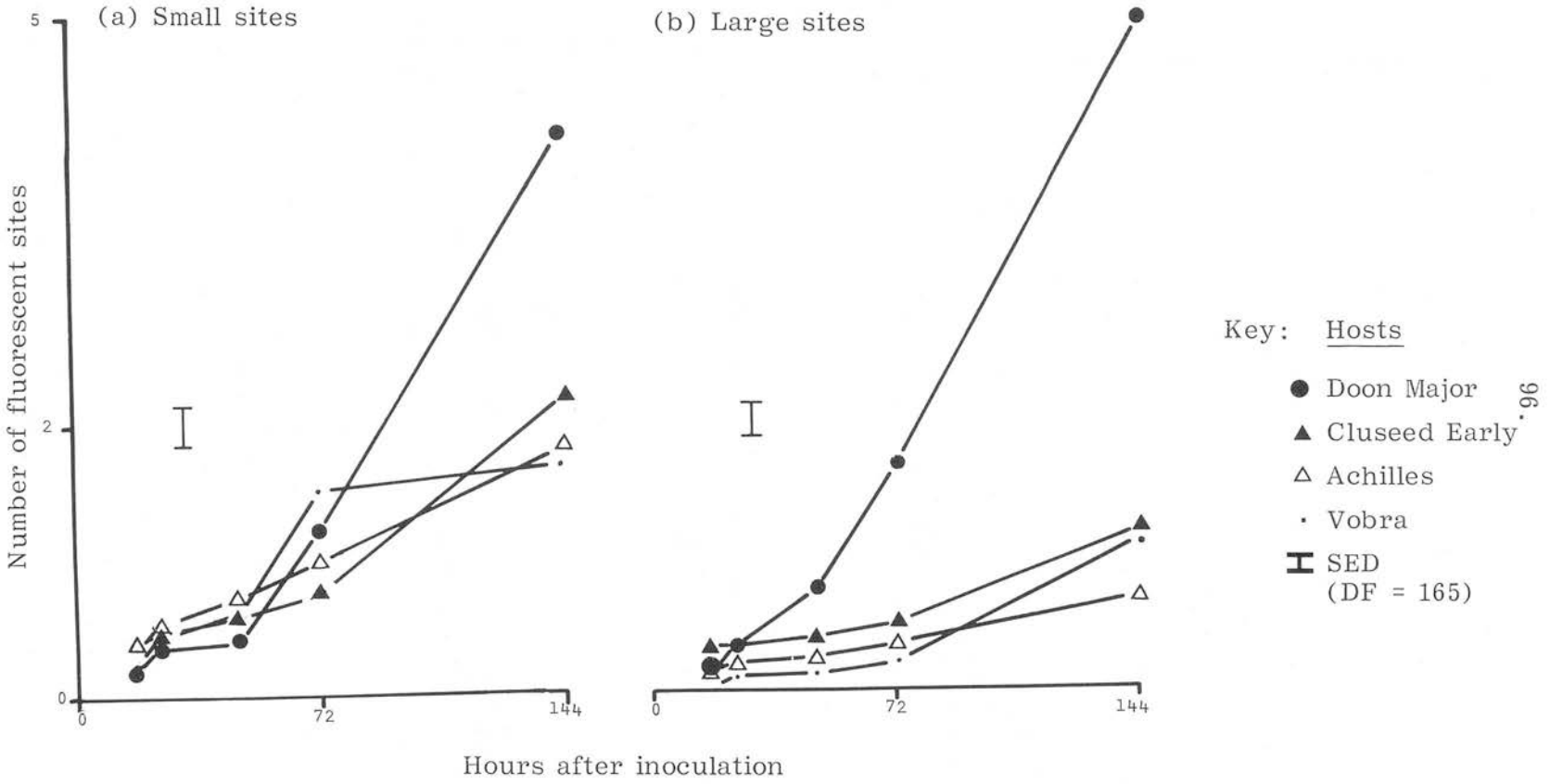


FIGURE 4.18: Number of small (<7 μm) and large (>7 μm) fluorescent sites associated with colonies of *E. cruciferarum* isolates on four *Brassica* hosts.

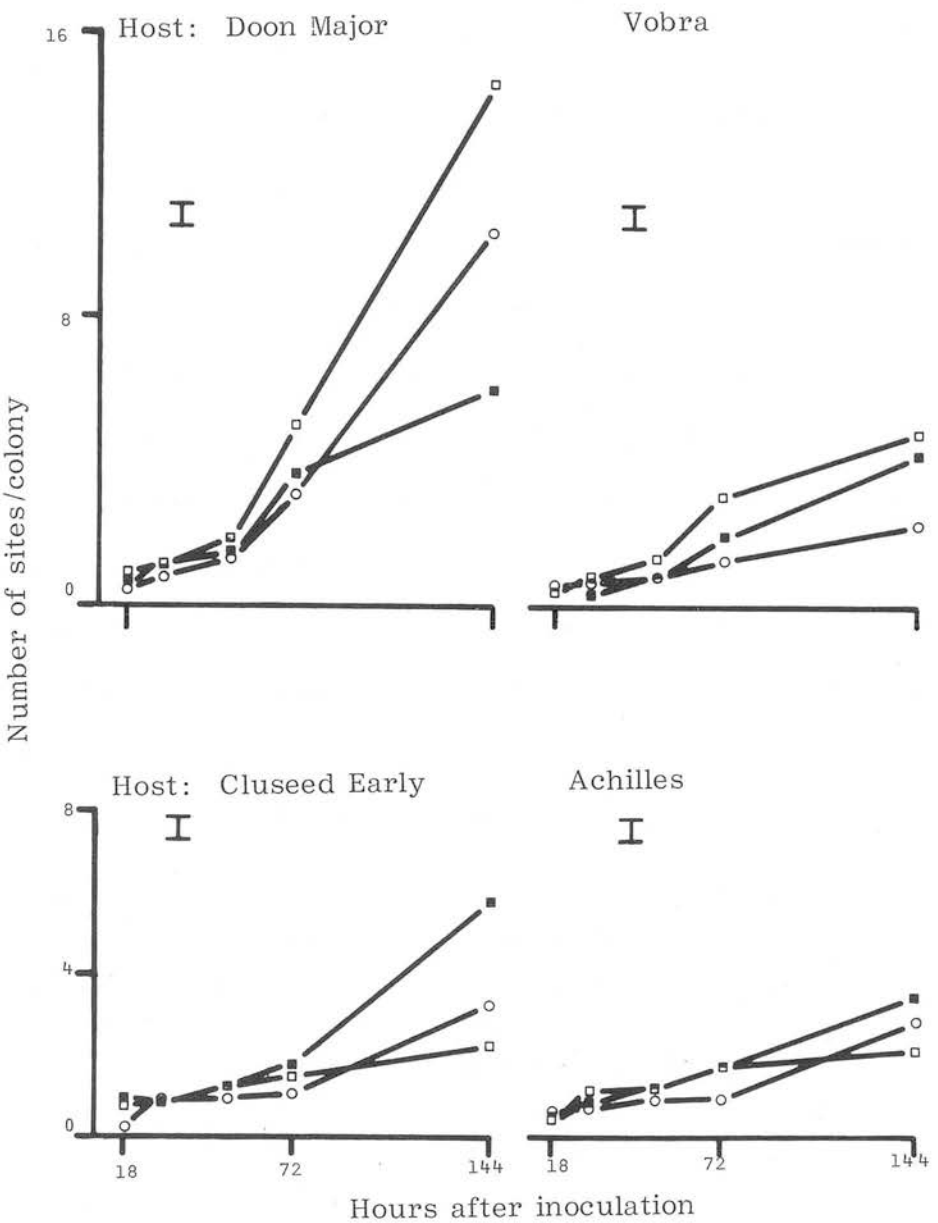


FIGURE 4.19: Total* number of fluorescent sites/colony of three *E. cruciferarum* isolates on four *Brassica* hosts.

*(large and small sites)

Key: Isolates

- □ N4a
- ○ N2e
- ■ O4a
- SED (DF = 165)

(v) Necrotic cell development

Necrotic cells were found 24 hours after inoculation on Vobra, Cluseed Early and Achilles, although at a very low frequency. On Doon Major cell necrosis was found at 48 hours. There was an overall increase in the numbers of necrotic cells with time.

Cell necrosis was first observed beneath primary A hyphae at 24 hours, beneath primary B and C hyphae at 48 and 72 hours respectively and beneath primary D and X hyphae only at 144 hours (Table 4.8). Most necrotic cells were found associated with primary A at each assessment time, and decreasing numbers at hyphal positions B, C, D and X in that order. No necrosis occurred on any of the uninoculated controls.

TABLE 4.8: General pattern of cell necrosis (overall mean frequency for all isolates and hosts).

Primary hyphal position	Hours after inoculation					SED ± (DF = 33)
	18	24	48	72	144	
A	-	0.02	0.08	0.15	0.64	0.02
B	-	-	<0.01	0.04	0.63	0.01
C	-	-	-	0.02	0.38	0.01
D	-	-	-	-	0.14	0.01
X	-	-	-	-	0.05	0.01

The number of necrotic cells varied significantly at all positions with host, but with isolate only at primary positions B, C and D. Significant interactions between host, isolate and time occurred beneath hyphae at all positions except X.

At all except the last assessment, cell necrosis occurred to a greater extent on Vobra, Cluseed Early and Achilles than on Doon Major

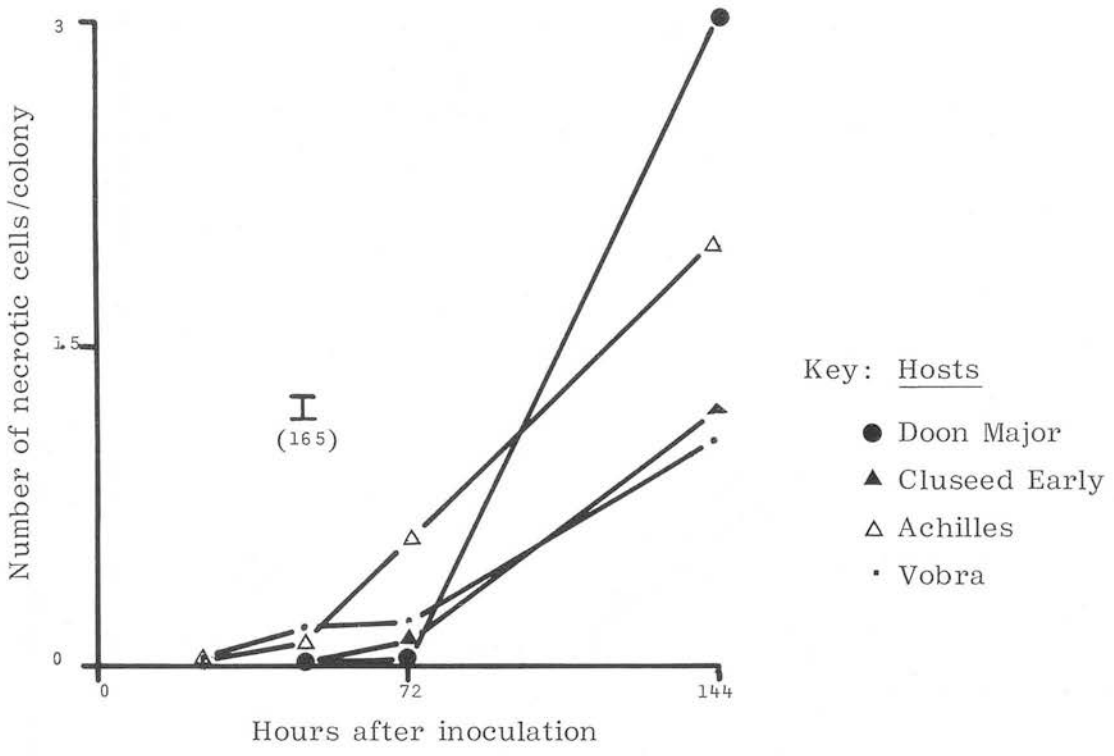


FIGURE 4.20: Number of necrotic cells beneath hyphal colonies of *E. cruciferarum* isolates on four *Brassica* hosts.

(Figure 4.20): at 24 and 48 hours, the frequency of cell necrosis was highest on Vobra but, at 72 hours most necrotic cells were found beneath colonies on Achilles. During the period of assessment up to 72 hours only very low levels of cell necrosis occurred on Doon Major: however, by 144 hours after inoculation, substantially greater numbers were found on Doon Major than on any other host.

Up until 72 hours after inoculation the frequency of cell necrosis did not differ significantly between isolates on any host (Figure 4.21). By 144 hours differences between isolates were evident, particularly with Doon Major which produced its highest number of necrotic cells in response to N4a, intermediate numbers beneath N2e colonies and fewest beneath O4a colonies. On Cluseed Early cell necrosis was higher beneath O4a and N2e colonies than beneath N4a colonies. On Achilles, numbers

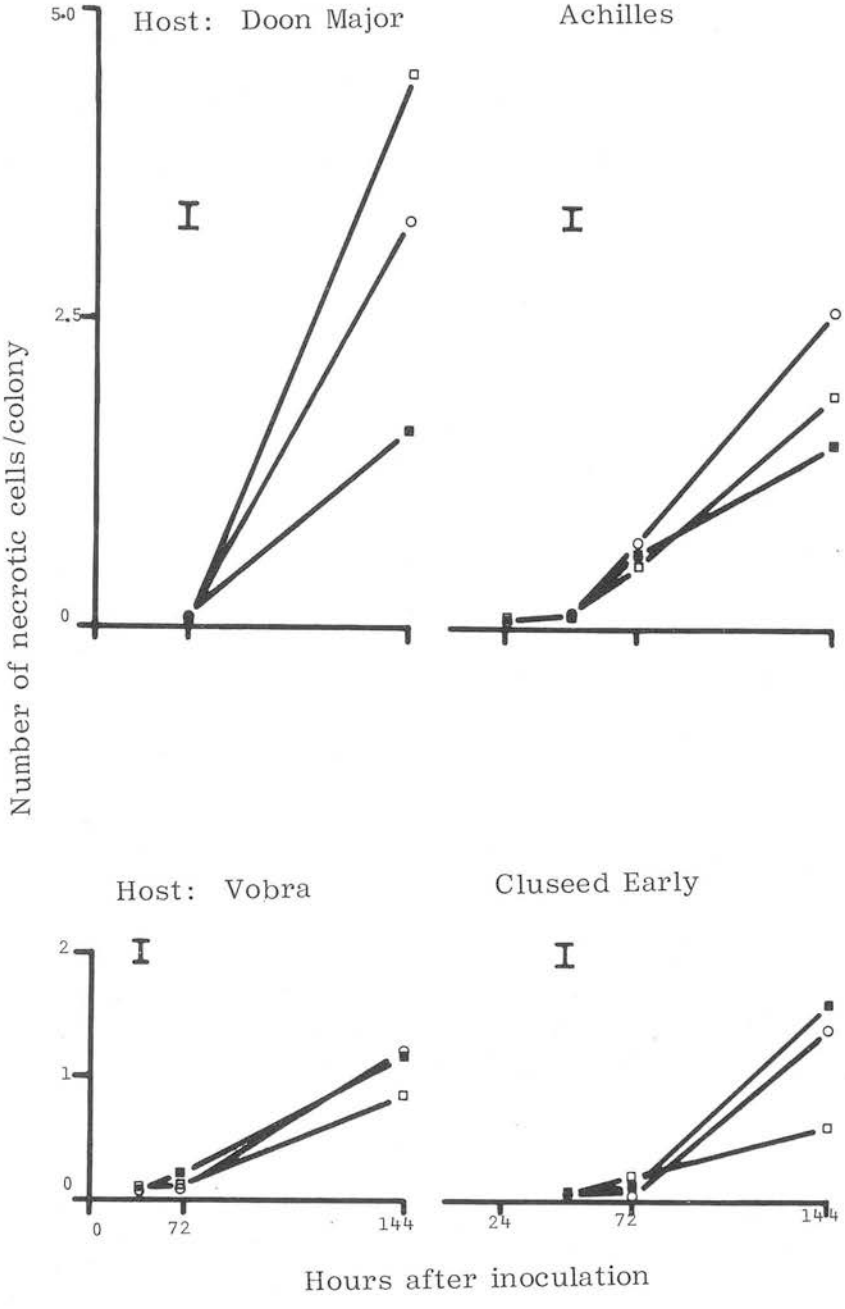


FIGURE 4.21: Number of necrotic cells/colony associated with three isolates of *E. cruciferarum* on four *Brassica* hosts.

Key: Isolate

- □ N4a
- ○ N2e
- ■ O4a
- H SED (DF = 165)

were highest beneath colonies of isolate N2e, intermediate beneath N4a colonies and least with O4a colonies. The differences in incidence of cell necrosis between isolates on Vobra were small, with N4a showing a slightly lower frequency than N2e and O4a.

Experiment 4.c: *Haustorial formation*

At 7 days, about four haustoria were observed on average with each colony and by 14 days the number had increased to seven in the microscope field of view (Table 4.9). At 7 days colonies were always fully within the microscope field of view but they had extended outside the field of view in some cases by 14 days. From 25 to 28% were categorised as abnormal haustoria for the two times of assessment. Overall the number of necrotic cells was low, with an average of only 0.6 and 1.0 necrotic cells per colony at 7 and 14 days respectively.

TABLE 4.9: Numbers of haustoria (normal and abnormal) and necrotic cells 7 and 14 days after inoculation.

	Haustoria (mean/colony)			
Time after inoculation	Total number	Normal	Abnormal	Necrotic cells (mean/colony)
7 days	4.4	3.3	1.1	0.6
14 days	7.2	5.2	2.0	1.0
SED ± (DF = 46)	0.15	0.11	0.08	0.04

The numbers of normal and abnormal haustoria, total number of haustoria and necrotic cell numbers were influenced by host, isolate and the different times as indicated in Table 4.10 and Figure 4.22.

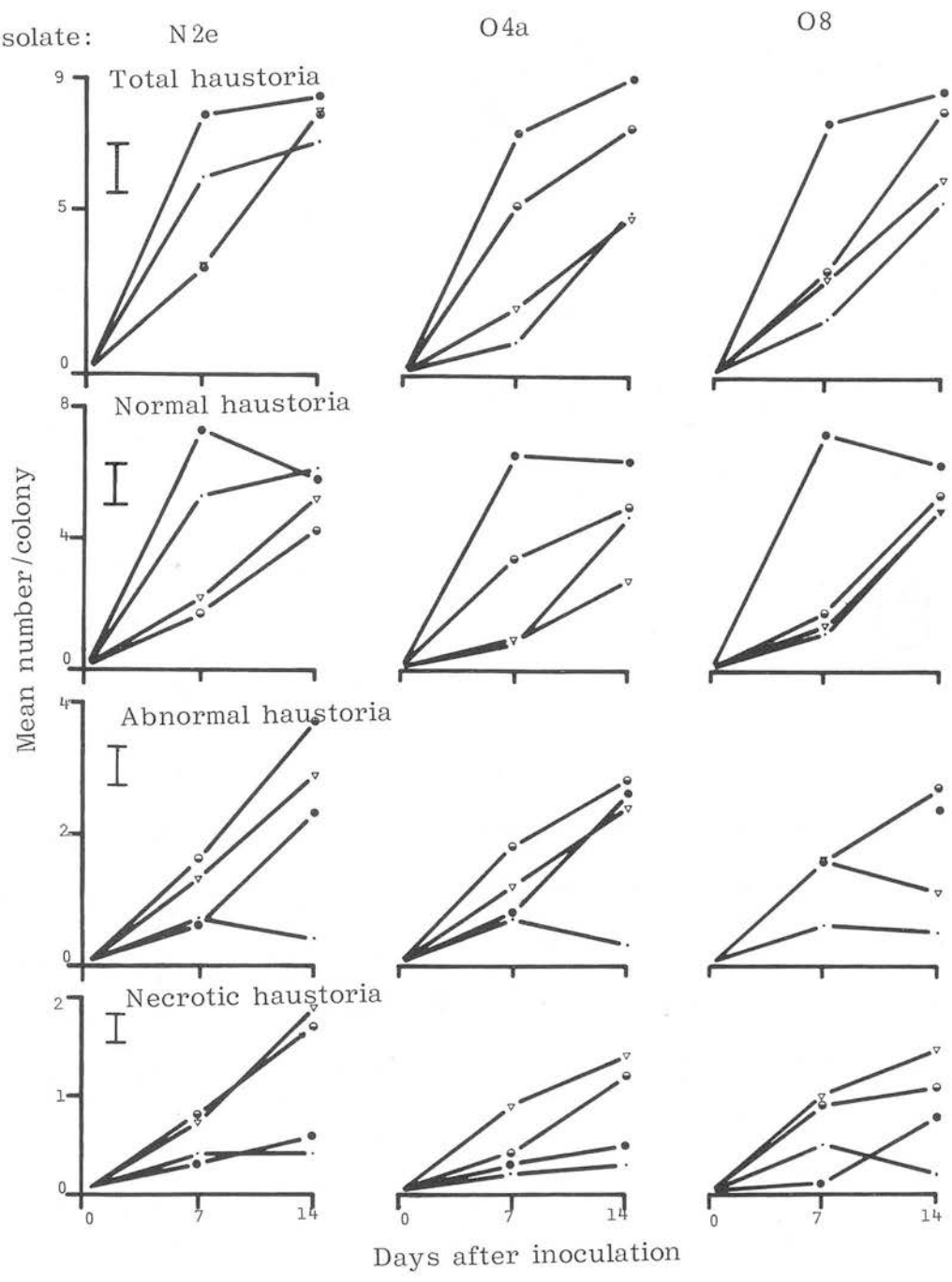


FIGURE 4.22: Number of necrotic cells, abnormal haustoria, normal haustoria and total number of haustoria below colonies of three *E. cruciferarum* isolates on four *Brassica* cultivars.

Key: Hosts

- ● Doon Major
- ◒ Lunet
- · Vobra
- ▽ Magres
- I SED (DF = 46)

TABLE 4.10: Mean number per colony of necrotic cells and haustoria (normal and abnormal) on four *Brassica* cultivars infected with *E. cruciferarum* isolates.

	Days after inoculation	Cultivar				SED ± (DF = 46)
		Doon Major	Lunet	Magres	Vobra	
Total haustoria	7	7.7	3.9	2.8	2.9	0.31
	14	8.8	7.9	6.3	5.8	
Normal haustoria	7	7.1	2.3	1.5	2.4	0.22
	14	6.4	4.9	4.2	5.4	
Abnormal haustoria	7	0.7	1.7	1.4	0.5	0.17
	14	2.4	3.1	2.1	0.4	
Necrotic cells	7	0.3	0.7	0.8	0.4	0.09
	14	0.6	1.3	1.6	0.3	

The total number of haustoria was greatest on Doon Major at both 7 and 14 days (8 and 9/colony respectively), intermediate on Lunet, and relatively low on Magres and Vobra (Table 4.10). The development of haustoria on Doon Major occurred mainly during the first 7 days after inoculation, after which colonies were extending outside the field of view: on other hosts the rate of increase in numbers of haustoria formed were similar during the first and second weeks.

The number of normal haustoria on Doon Major was maximum at 7 days and declined by 14 days as numbers of abnormal haustoria increased. However, on Vobra, Magres and Lunet the number of normal haustoria increased between 7 and 14 days (Table 4.10). The numbers of abnormal haustoria were higher on Magres and Lunet than on other hosts at 7 days and, as with Doon Major, increased from 7 to 14 days. Vobra was associated with a relatively low incidence of abnormal haustoria and numbers did not increase over the observation period. The per-

centage of total haustorial numbers which were abnormal was lower after 14 days on all hosts except Doon Major, on which there was a substantial increase.

Cell necrosis incidence increased on all cultivars except Vobra from 7 to 14 days. Numbers of necrotic cells were very low on this cultivar and also on Doon Major, relative to the incidence on Magres and Lunet.

There was little difference between the behaviour of the different isolates on Doon Major but isolate differences were found on all other hosts (Figure 4.22). On Vobra, isolate N2e produced normal haustoria more rapidly and to a greater extent than either O4a or O8. The numbers of abnormal haustoria and necrotic cells found on Vobra for colonies of each isolate were more or less similar. The number of normal haustoria produced on Magres was greater with colonies of N2e and O8 than with O4a, but more abnormal haustoria were produced by N2e and O4a: necrotic cells were eventually found more frequently with N2e than with other isolates. The three isolates produced, by 14 days, similar total numbers of haustoria on Lunet, but isolate N2e had fewer normal haustoria and greater numbers of abnormal; in addition, N2e gave rise to more host cell necrosis than either O4a or O8 (Figure 4.22).

DISCUSSION

The variation in the level of powdery mildew infection on different cruciferous hosts, indicated in an earlier experiment (Experiment 3.a), was again evidenced in the more detailed studies on fungal colony development in this section. Doon Major was shown to be the most susceptible host and all isolates of *E. cruciferarum* produced generally more rapid and extensive colony development on this cultivar than on any other host. Using a similar Fungal Development Scale to that used in the present study, Brain (1978) showed the proportion of conidia of *E. cruciferarum* reaching the expanding colony stage at 48 hours varied considerably on different cultivars of *Brassica* species (*B. napus* and *B. oleracea*), reflecting their differences in susceptibility to infection. The effect of host on fungal development emerged from the primary hyphal stage onwards (Figures 4.3 and 4.9). In keeping with other reports (White and Baker, 1954; Lupton, 1956; Moseman, Scharen and Creeley, 1965), rates of germ tube and appressorium formation did not appear to be influenced greatly by the hosts used, which included, in Experiments 4.a, .b and .c as a whole, members of *B. campestris*, *B. carinata*, *B. napus*, *B. oleracea*, *Raphanus* and *Raphanobrassica*.

Fungal development was affected at various stages after appressorium formation on more resistant hosts. In some instances the majority of infections did not proceed beyond the appressorial stage, *viz*. isolate N4a/BC82 (*B. carinata*), N4a/Barsica (*B. napus*), N4a/RB25/8 (*Raphanobrassica*) and N2e/RS15 (*R. sativus*) (Figure 4.4). In some cases, however, resistance was associated with a reduced development beyond the primary or secondary hyphal stage, e.g. isolate N2e with hosts RB25/8 and BC82 and isolate N4a with RS15 (Figure 4.4), and with

three isolates N2e, N4a and O4a on Vobra (*B. campestris*), Achilles and Cluseed Early (*B. oleracea*) (Figure 4.10). However, with isolate O4a on Cluseed Early, those colonies that did develop beyond the secondary hypha stage were relatively vigorous (Figure 4.23) with a substantial proportion reaching the conidiophore stage by 144 hours. The restricted colony development usually associated with Vobra, Cluseed Early and Achilles compared with fungal growth on Doon Major is illustrated in Figure 4.23.

The retarded and reduced colony growth on more resistant hosts was reflected in a reduced development of spore producing colonies over the period of observation in experiments. Thus, in Experiment 4.b at 96 hours after inoculation, 80 to 100% of colonies, depending on isolate, were at the conidiophore initial stage on Doon Major but the proportions for other cultivars ranged from 0 to only 30%. In Experiment 4.c, at 144 hours after inoculation, the percentage of colonies initiating conidiophores or producing conidia varied from 17 to 33% for the three isolates on Doon Major and from 0.5 to 28.5% for the same isolates on other hosts (Table 4.6).

From these results it is seen that host resistance to mildew may be expressed as reduced mycelial growth and spore production. Clifford (1975) and Brain (1978) considered that the resistance of Ruta Otofte (*B. napus*) to *E. cruciferarum* was due to a restriction of colony extension and of re-infection of new sites.

In the experiments on host resistance in this section, 95% or more of the conidia had germinated on all hosts by 24 hours after inoculation, indicating a high infection efficiency (Ellingboe, 1972). Moreover, on the susceptible host, Doon Major, the synchrony of parasite units of any isolate was high and the inter-relationship between *E. cruciferarum*

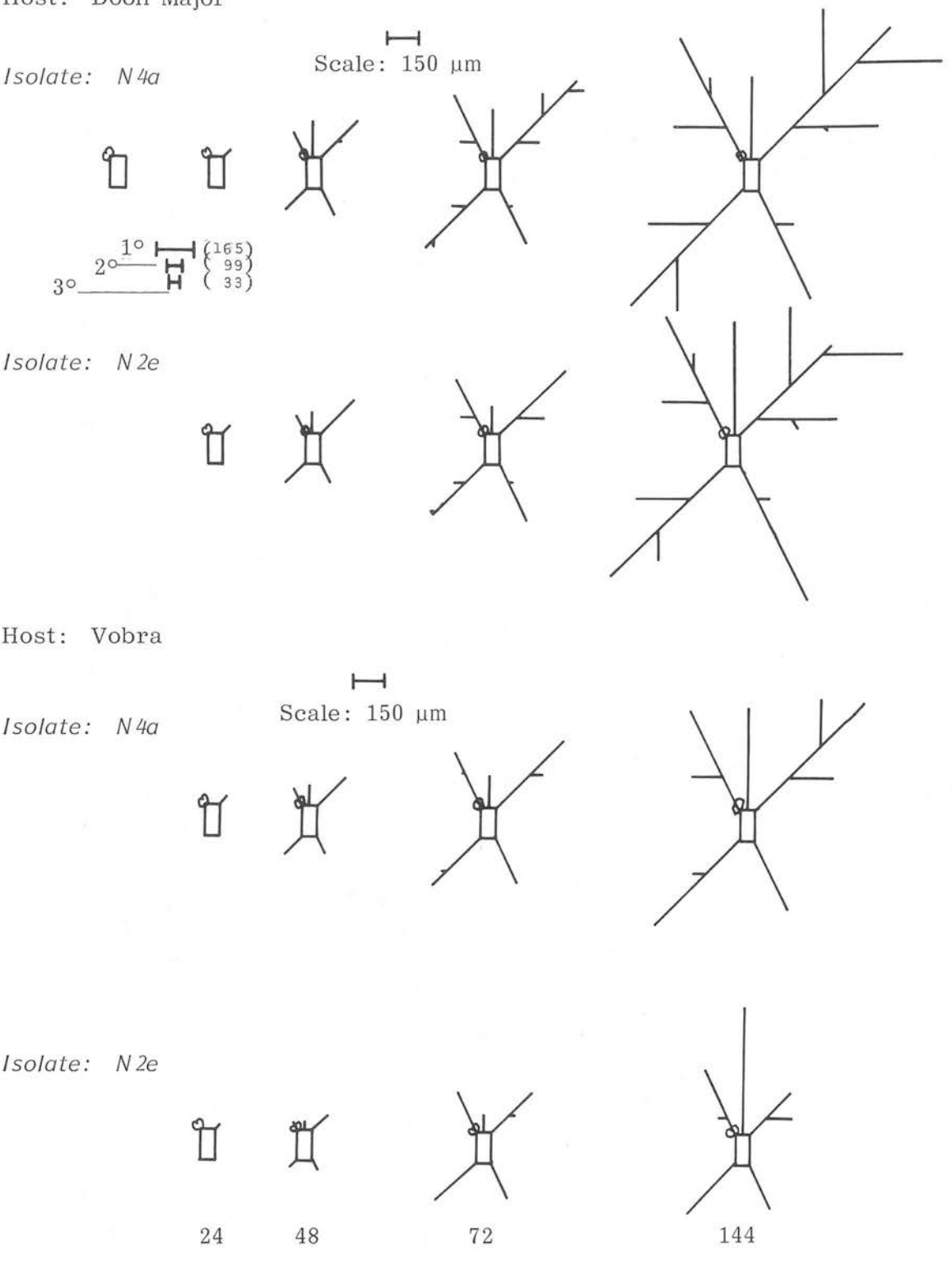
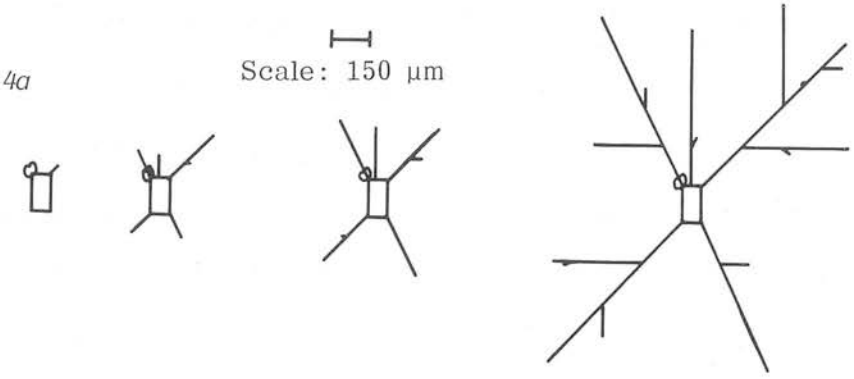


FIGURE 4.23: Diagrammatic representation of colony development of *E. cruciferarum* isolates on four *Brassica* cultivars. (Isolates showing maximum and minimum development on each host are selected for illustration.)

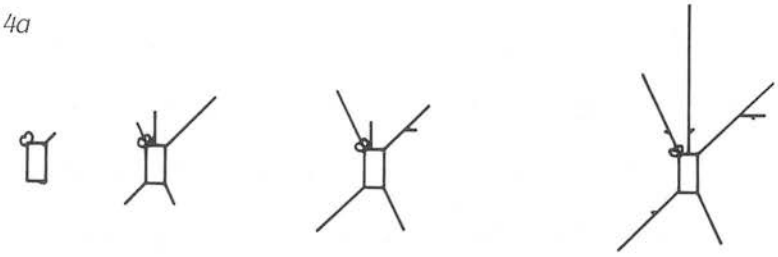
Host: Cluseed Early

Isolate: O4a

Scale: 150 µm



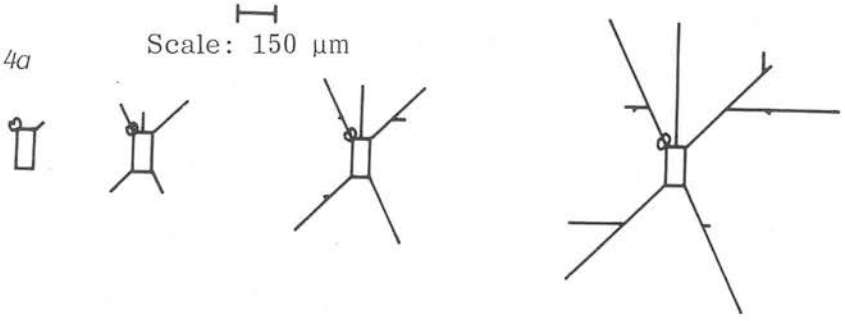
Isolate: N4a



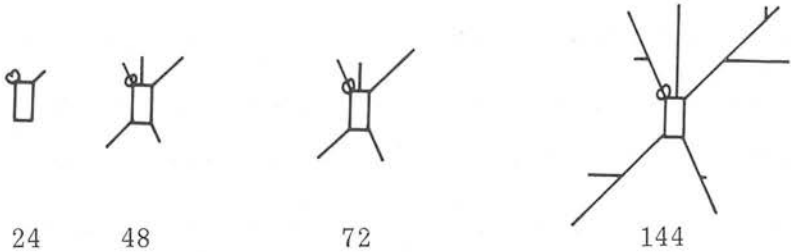
Host: Achilles

Isolate: O4a

Scale: 150 µm



Isolate: N2e



24 48 72 144

Figure 4.23: (continued)

and Doon Major conforms to the compatible isolate/host combination discussed by Ellingboe. The high infection efficiency and the high degree of synchrony allow the expression of interaction between other host and isolate genotypes to be assessed. Therefore, while all isolates developed to a more or less similar extent on Doon Major, some interactions between isolates and other hosts were evident in Experiments 4.b and 4.c, suggesting some degree of host specificity. As previously indicated, in Experiment 4.b, isolate N4a showed an incompatible, and N2e a compatible relationship with Barsica, whereas the combination N2e/RS15 was less compatible than N4a/RS15 (Figure 4.4). In Experiment 4.c differential responses of isolates on different hosts were less clear cut. The results suggest, as in the previous section, that the variation in isolate/host responses are quantitative rather than qualitative and there was an infrequent occurrence of host specificity among isolates expressed in well defined categories.

Compared with Doon Major, all hosts tested exhibited a degree of resistance expressed at a complete or partial level. Complete resistance prevented growth beyond the appressorium stage, but was never found to occur with all infection units. Thus, with the cultivars Barsica, BC82, RB25/8 and RS15, on which a large proportion of infection units of certain isolates failed to produce hyphae, small numbers of conidia on leaves still showed further colony development. Hsu and Ellingboe (1972) observed that even with incompatible *E. graminis* isolate/cereal host combinations a percentage of infection units always succeed in attaining maximum development. The complete form of resistance, however, is comparable with the first major expression of resistance of cereals to mildew (Masri and Ellingboe, 1966; McCoy and Ellingboe, 1966; Shaner, 1973; Lin and Edwards, 1974; Carver and Carr, 1977). Resistance of

this type prevents the establishment of primary infection and reduces the numbers of colonies which develop from a standard level of inoculum.

Partial resistance emerged in this study as causing a delay or restriction in the progressive development of colonies and a reduced sporulation capacity of colonies. Consequently, the rate of pathogen spread would be decreased, a phenomenon observed by Brain (1978) during studies of field infection of *Brassica* cultivars by *E. cruciferarum*. Partial resistance has been reported to contribute to the slow-mildewing of wheat (Shaner, 1973). In 1983, Asher and Thomas examined the components of partial resistance of spring barley cultivars to *E. graminis* and indicated that both reduced colony numbers and reduced colony growth rate were implicated, reflecting the complete and partial resistance described in the present studies.

Based on Ellingboe (1972), Day (1974) gave a diagrammatic representation of points at which major genes for resistance in wheat and barley block early development of powdery mildew infection. In attempting a similar diagram for the ontogeny of interaction of *E. cruciferarum* isolates on different cruciferous hosts, a range of resistance expressions is shown (Figure 4.24). Ellingboe's studies were with hosts possessing recognised major genes for resistance. From the few genetical analyses of cruciferous plants (Walker and Williams, 1965; Brain and Whittingdon, 1980), resistance to *E. cruciferarum* has been shown to be controlled by an incompletely dominant gene influenced by many minor genes with small additive effects. This finding is in keeping with the quantitative variation in isolate/host response shown in the present work.

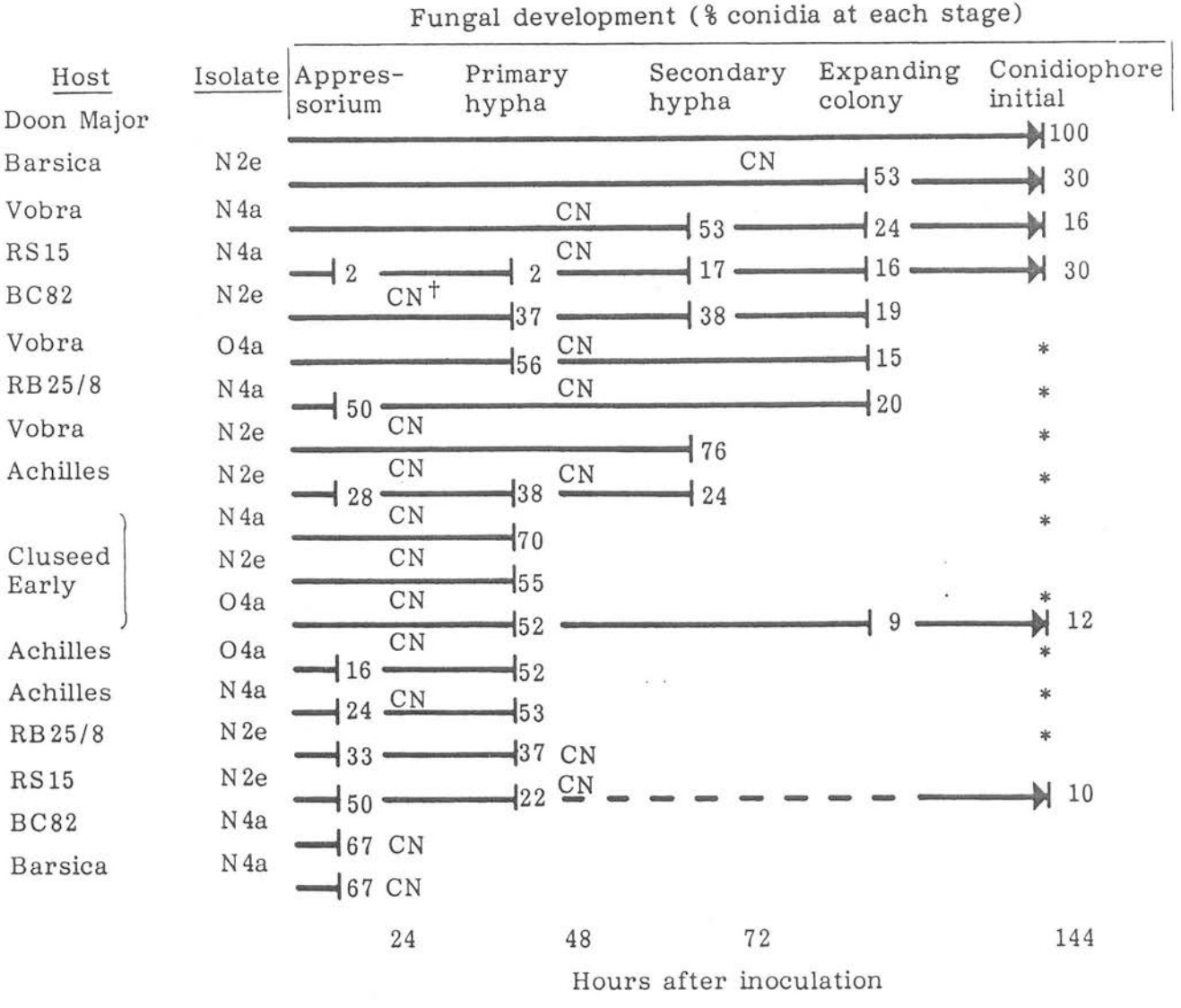
Although the results indicate that the resistance in cruciferous plants to powdery mildew tends to be of a generalised and possibly of a non-specific nature, as described by Shaner (1973), Carver and

FIGURE 4.24:

Effect of resistance of different cruciferous hosts on development of *E. cruciferarum* isolates.

† CN = First observation of cell necrosis.

* Less than 10% of conidia reached sporulation stage.



Carr (1977) and Bennett (1981), resistance in certain hosts affected the development of isolates differently (Figures 4.4, 4.10 - 4.15). Although generalised and partial resistance mechanisms are often considered effective against all races of a pathogen equally, differential interaction between host and pathogen with polygenic or background resistance have been recorded (Parlevliet, 1977; Wolfe and Schwarzbach, 1978). In 1978, working with a number of isolates of *Puccinia hordei* and cultivars of barley, Parlevliet reported further evidence of the resistance conferred by polygenes being overcome by a particular isolate, which developed more rapidly than all other isolates on a particular cultivar. In the present study, some variation in the responses of different isolates on particular hosts showing some degree of resistance was evident. Without a genetical analysis of the specificity of each host tested, however, no positive conclusions can be put forward as to the nature of the genetical control of resistance or pathogenicity.

In considering host tissue responses to infection by *E. cruciferarum*, two events which occurred frequently were the development of fluorescent sites and cell necrosis. Invariably the susceptible cultivar Doon Major showed the greatest number of fluorescent sites from each infection unit, in comparison with other hosts. In these studies, aniline blue was used especially to identify callose deposits. It has long been known that callose is deposited between the plasma membrane and cell wall in response to mechanical injury (Currier, 1957) and the number of fluorescent callose sites may be considered to reflect the number of attempted penetrations. Thus, increasing mildew development will be expected to give rise to increasing numbers of fluorescent sites, i.e. penetration points, and this is shown to be the case in Experiment 4.a as illustrated in Figure 4.25. The results of Experiments 4.b and 4.c confirm the

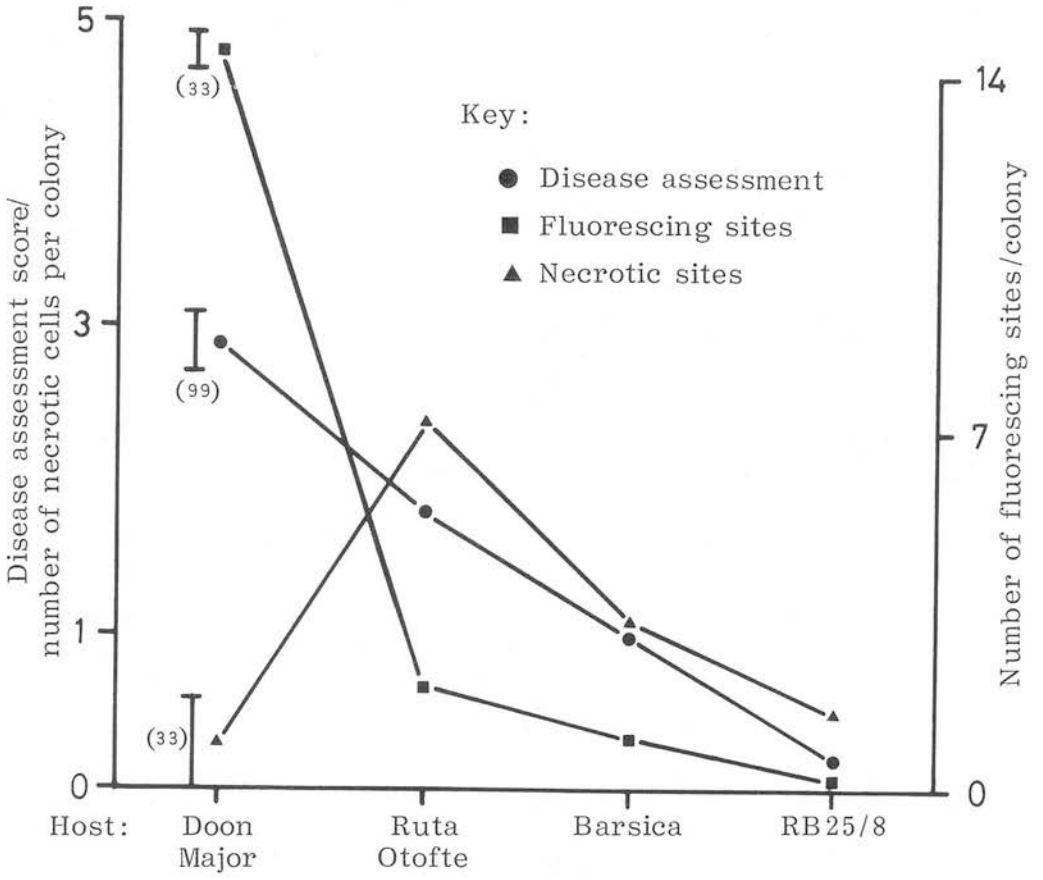


FIGURE 4.25: Development of *E. cruciferarum*, number of fluorescing sites/colony and number of necrotic cells/colony on four cruciferous hosts 120 hours after inoculation. (Mean of three isolates)

relationship between colony growth and numbers of fluorescent sites when assessments were carried out over a period of time (Figure 4.26). Callose deposition was found on all hosts and is considered to be a generalised response by hosts to attempted penetration, as observed by Eschrich and Currier (1964) and Maclean and Tommerup (1979). Thus, more susceptible hosts showed greater numbers of callose sites as a consequence of increased mycelial development and associated frequency of penetrations. Moreover, in time course studies, callose deposits were observed earlier on the susceptible Doon Major than on resistant hosts. As rates of germination and of appressorium formation

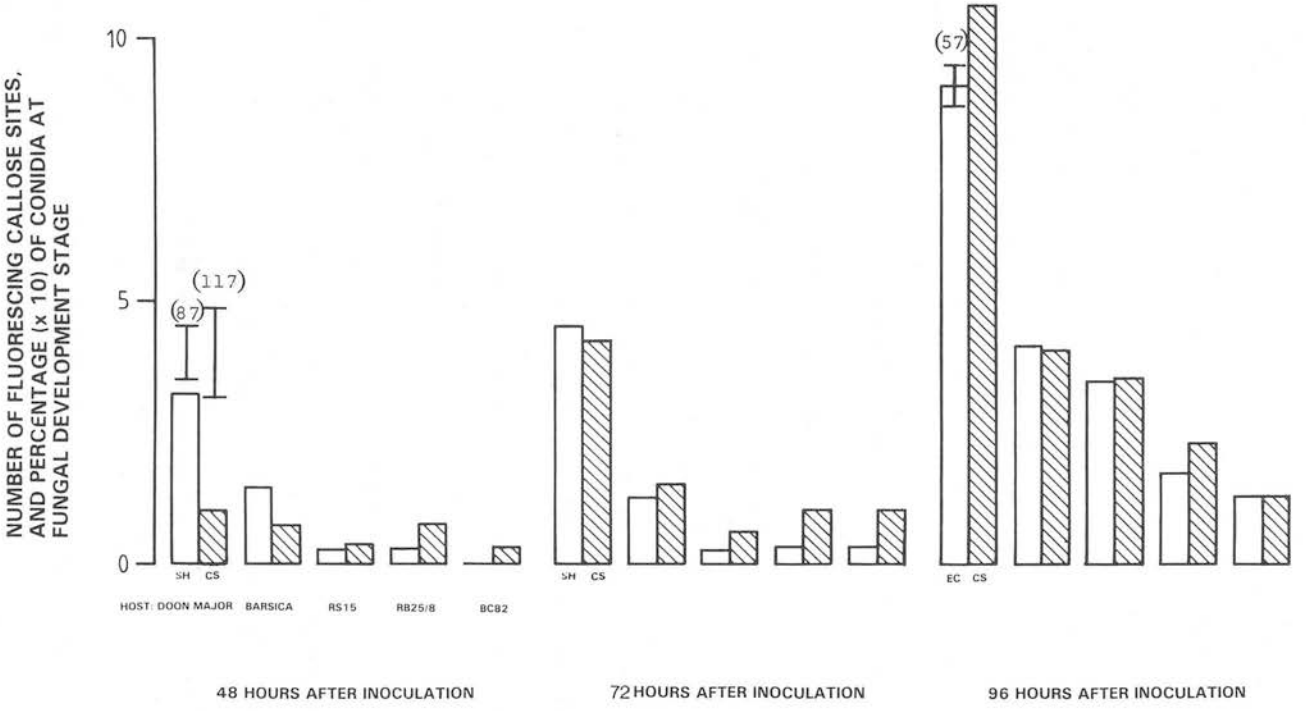


FIGURE 4.26: Number of fluorescing callose sites (CS) and percentage of conidia of *E. cruciferarum* isolates at the secondary hyphal (SH) or expanding colony stage (EC) of development on five cruciferous hosts at different times after inoculation.

were the same on all hosts, delayed callose deposition on more resistant hosts may reflect delayed completion of penetration, suggesting a component of resistance in the cuticle. However, some workers (Mence and Hildebrandt, 1966; Ellingboe, 1976) have implied resistance to mildew operates only after penetration.

There are many reports of cell necrosis of resistant host tissues in response to infection (Maclean, Sargent, Tommerup and Ingram, 1974; Shimony and Friend, 1974; Skipp and Samborski, 1974; Heath, 1976) and the relationship between early cell necrosis (hypersensitivity) and resistance is widely, although not universally, accepted. In the present work (Experiments 4.a, .b and .c) varying relationships between the extent of cell necrosis and degree of host resistance, depending upon the particular host and time after inoculation when observations were made. In Experiment 4.a, necrotic cell number was low in the most susceptible cultivar, Doon Major, but increased with increasing fungal development for other hosts (Figure 4.25). Doon Major/*E. cruciferarum* may be considered a compatible combination of host and biotroph, where infection cells are maintained alive over an extended period. With the other cultivars, the greater the degree of resistance the fewer the number of cells affected by a necrotic response. With cereal mildew, resistance has been associated with death of infected epidermal cells (Cherewick, 1944) or by mesophyll collapse (White and Baker, 1954). In the latter case the degree of resistance was considered a function of the rapidity of response: with higher levels of resistance collapse occurred earlier and was confined to fewer cells. A similar type of relationship between the rate and extent of development of dead epidermal cells and resistance to powdery mildew infection is suggested for the cultivars Ruta Otofte, Barsica and RB25/8 (Figure 4.25). From studies

on the appearance of necrosis in relation to time (Experiments 4.b and 4.c), hosts which gave rise to more cell necrosis (Figures 4.6 and 4.20) during the earlier stages of infection tended to be associated with less fungal development (Figure 4.5 and 4.19). This negative correlation is clearly illustrated in Figure 4.27.

In considering the behaviour of two test isolates on Barsica in Experiment 4.b, with the incompatible combination of N4a/Barsica cell necrosis was first found 24 hours after inoculation, compared with 72 hours for the compatible N2e/Barsica relationship. Cell necrosis was always more frequent with the incompatible combination but hyphal development less (Figure 4.28). Heath (1982) and Kluczewski and Lucas (1982) considered that events which ultimately result in cell death were initiated much earlier in the more resistant hosts, thereby disturbing the nutritional relationship with living cells required by biotrophs. From these studies, at least in some instances, cell necrosis is concluded to play a role in the resistance of cruciferous hosts to infection by *E. cruciferarum*, the more rapid the response the more effective the restriction of infection.

In Experiment 4.c, where the period of observation was longer than in the previous experiments, the highest numbers of necrotic cells were finally recorded on the susceptible Doon Major, at the time when colony growth and the development of spore producing structures were extensive on this host. In this case cell necrosis did not relate to resistance but, as observed by Peries (1962b) and Kluczewski and Lucas (1982), more to cell exhaustion as a consequence of the demands of the fungus.

Several workers (White and Baker, 1954; Masri and Ellingboe, 1966) have shown there to be a good correlation between elongating

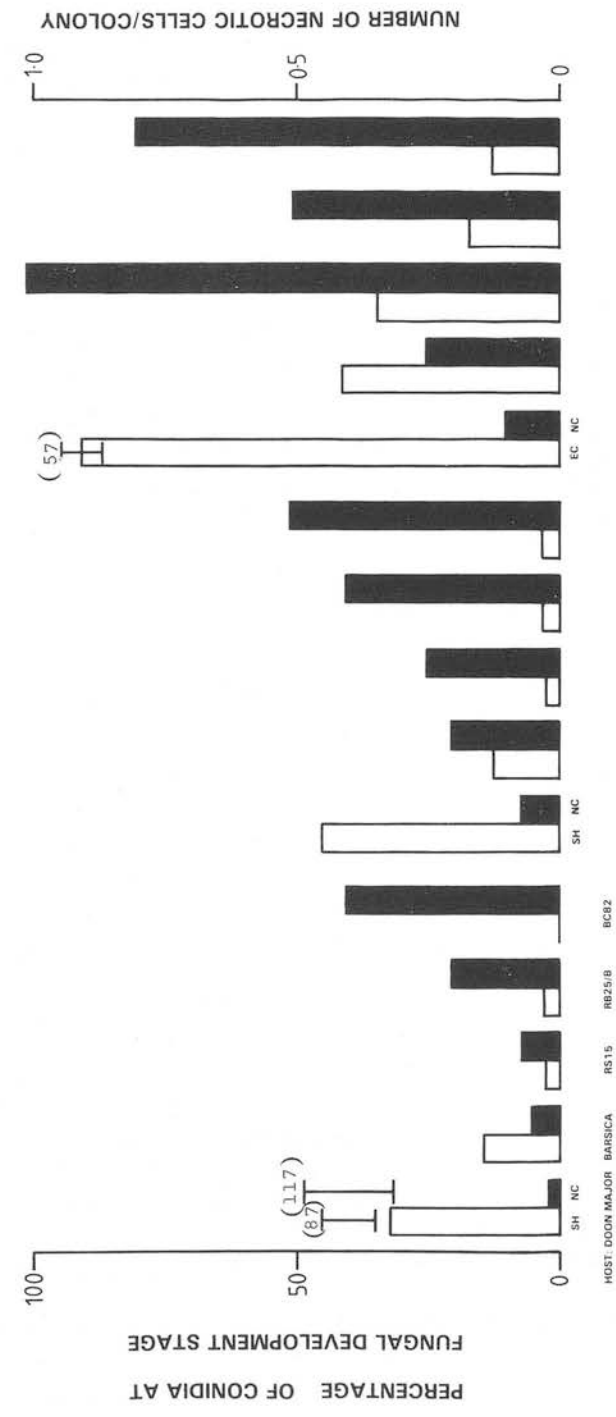


FIGURE 4.27: Number of necrotic cells (NC) and percentage of conidia of *E. cruciferarum* isolates at the secondary hyphal (SH) or expanding colony stage (EC) of development on five cruciferous hosts at different times after inoculation.

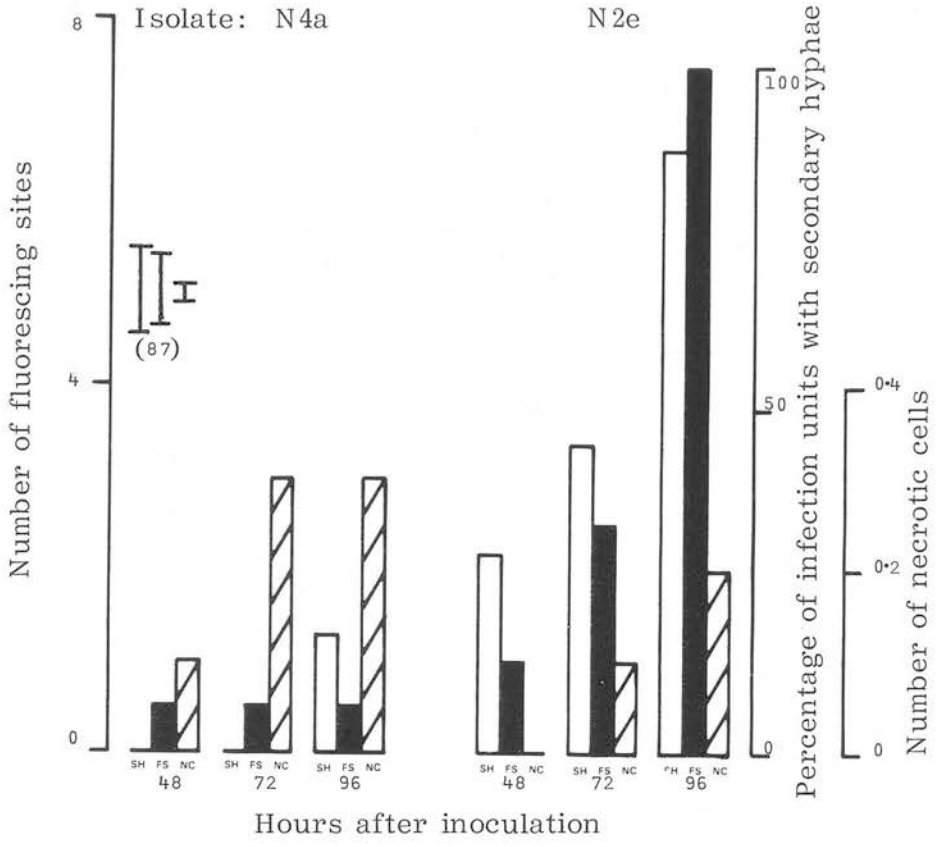


FIGURE 4.28: Percentage of conidia with secondary hyphae (SH), number of fluorescent sites/colony (FS) and necrotic cells/colony (NC) of two isolates of *E. cruciferarum* on the host Barsica.

secondary hyphae and the formation of functional haustoria. By showing that colony development ceased soon after the excision of haustoria, Bushnell (1971) confirmed that the primary determinant of compatibility is the establishment of active haustoria. From the Experiments 4.a, 4.b and 4.c, in which the numbers of fluorescent sites per colony are considered to represent the numbers of penetration attempts per colony, fluorescent site number may be considered as an estimate of the possible numbers of haustoria per colony. In Figure 4.29, the number of fluorescent sites per colony of each isolate/host combination in Experiment 4.c is shown along with the number of necrotic cells per colony. The dif-

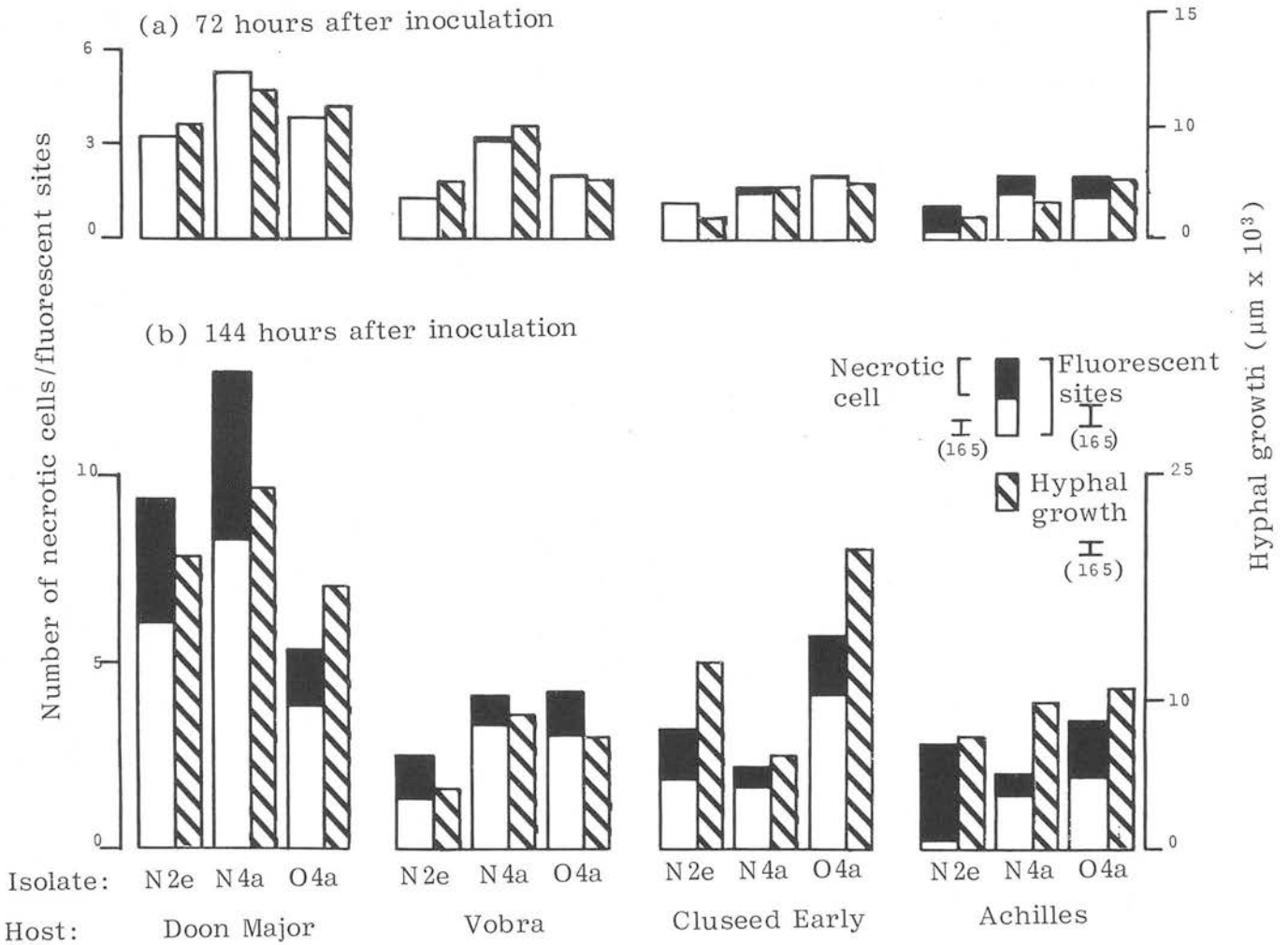


FIGURE 4.29: Number of necrotic cells, fluorescent sites and hyphal growth per colony of three *E. cruciferarum* isolates on four *Brassica* hosts.

ference between levels of these two factors (the unshaded area of the histogram) may be regarded as representing the possible number of penetration sites with a functional haustorium per colony. A high correlation was found between this estimate of functional haustoria per colony and colony growth (correlation coefficient r = 0.91, Df = 23).

The last experiment in this section, Experiment 4.d, aimed to examine haustorial development on cruciferous hosts varying in susceptibility to *E. cruciferarum*. The results of the investigation showed that the rate of development of haustoria varied on different hosts. Twice as many haustoria were produced during the first 7 days on Doon Major than on any of the other hosts, increased numbers of haustoria per colony reflecting increased susceptibility as previously reported by White and Baker (1954).

Host resistance has been related not only to reduced haustorial numbers, but also to reduced haustorial efficiency which can seriously affect fungal growth (Heath, 1982). Mence and Hildebrandt (1966) found a higher proportion of abnormal haustoria (either collapsed or degenerate) associated with colonies on more resistant hosts. Haustorial disorganisation has been associated with resistance by Smith (1938) and Carver and Carr (1978), while Heath (1982) considered the timing of haustorial disorganisation to be critical, in that, if a haustorium can function even briefly, the fungus may obtain sufficient nutrients to allow further growth and additional attempts at haustorial formation. In the present study, there were much larger percentages of haustoria which were abnormal at 7 days after inoculation on the more resistant hosts Lunet and Magres, 40 and 46% respectively, than on the susceptible Doon Major with only 8% abnormal haustoria (Table 4.10). Although the precise timing of haustorial disorganisation was not recorded, the

presence of abnormal haustoria which are likely to be either non-functional or have impaired functioning, will be expected to reduce colony development accordingly (Masri and Ellingboe, 1966). On Doon Major, the number of abnormal haustoria per colony increased over the period 7 to 14 days after inoculation. In agreement with Mence and Hildebrandt (1966), this development of abnormal haustoria from colonies on Doon Major would appear to be part of the ageing process in older parts of colonies as they expand and host cells senesce: normal haustoria may be expected to occur in the advancing regions of colonies while earlier formed haustoria would be expected to degenerate with time.

5. HISTOCHEMICAL STUDIES OF HOST CELL RESPONSES TO INFECTION BY *E. CRUCIFERARUM*

INTRODUCTION

In the previous section the main interest related to the pattern and extent of fungal development in various combinations of *E. cruciferarum* isolates and cruciferous hosts, which expressed varying levels of compatibility or host susceptibility/resistance. Various changes in host cells in response to infection were also observed and these are considered further in the present section along with their possible significance as resistance factors.

The occurrence of fluorescent sites, associated with callose deposition, was found to be more frequent with more extensive fungal development in keeping with the findings of Maclean and Tommerup (1979) who found a relationship between compatibility and increased callose production. Attempted penetration of the leaves of wheat and other members of the family Gramineae by fungi is frequently accompanied by the formation of papillae (Stanbridge, Gay and Wood, 1971; Bushnell and Berquist, 1975) and a densely stained 'halo' around the penetration point on the epidermal cell wall (Kunoh, 1972). Papillae are formed following halo initiation, and are deposited as hemispherical appositions between the plasmalemma and cell wall (Sherwood and Vance, 1982). Histochemical tests have indicated the presence of callose and lignin in haloes and papillae (Sherwood and Vance, 1976; Ride and Pearce, 1979). Lignification at penetration sites has been associated with resistance. However, in studies where lignification has been detected the fungal species were inoculated onto non-hosts (Ride, 1980). Lignin was not detected at infection sites in resistant barley inoculated with *E. graminis* f. sp. *hordei*, nor in resistant oat leaves inoculated with *Pyrenophora teres* (Hargreaves, 1982).

Following inoculation of different hosts by pathogenic fungi, fluorescing material has been revealed in both papillae and haloes. This material was identified as callose in barley (Stanbridge *et al.*, 1971) and in lettuce (Maclean and Tommerup, 1979) and of a polyphenolic nature in oats (Kidger and Carver,1981) and barley (Mayama and Shishiyama, 1978). Callose was detected by aniline blue and resorcinol blue, both of which are specific for callose (Currier, 1957; Eschrich and Currier, 1964). Hypersensitively collapsed epidermal cells have been identified by their intense fluorescence following aniline blue staining treatment (Mayama and Shishiyama, 1978; Koga *et al.*, 1980).

A study by Koga *et al.* (1980) compared the development of different races of *E. graminis* f. sp. *hordei* on four varieties of barley. As host resistance increased, the greater was the rate of cessation of fungal growth at the papilla stage, the stronger was the fluorescence intensity of papillae and the greater was their size. Haloes were larger in susceptible (40 μm) than in resistant hosts (20 μm), although they generally did not form where haustoria were produced. In susceptible barley papillae were small and fluoresced weakly when associated with haustoria. Koga *et al.* (1980) suggested that compatibility or incompatibility are expressed at the papilla stage and the fluorescent compounds deposited at the infection sites might represent chemical factors which are associated with resistance expressed at penetration.

The presence of polyphenolic substances in papillae on barley leaves inoculated with *E. graminis* f. sp. *hordei* was confirmed (Mayama and Shishiyama, 1978) by the positive reactions obtained after the diazobenzene sulphanilic acid and diazotised p-nitro aniline tests (Suzuki, 1962).

The experimental work in the present section considers the changes in host cells in response to infection, in particular, callose deposition, the production of lignin and lignin aldehydes and of phenolic compounds. The investigations are considered under the following headings:

5.a Callose deposition
  (i) Leaf surface studies
  (ii) Transverse section studies

5.b Lignin and lignin aldehyde formation [(i) and (ii)]

5.c Phenolic compound production

MATERIALS AND METHODS

Experiment 5.a(i)

Cultivars Doon Major and Barsica (*B. napus*), Achilles and Cluseed Early (*B. oleracea*), Vobra (*B. campestris*), breeding lines RB25/8 (*Raphanobrassica*) and BC82 (*B. carinata*) were grown in a glasshouse (Section 2) and samples of leaf material were inoculated with isolates N2e and O4a. Inoculum of each isolate was prepared by method B; leaf discs were inoculated and incubated as described in Section 2. Assessments were made 8, 12, 18, 24, 48, 72, 120 and 144 hours after inoculation.

At each time of assessment inoculated leaf discs, and uninoculated controls at the final assessment, were transferred to 70% ethanol for clearing in frequent changes of alcohol. Following clearing, three replicate leaf discs were immersed in aniline blue solution (Section 2) for 24 hours and the other three were placed into resorcinol blue stain (O'Brien and McCully, 1981) for 4 hours, then rinsed in distilled water and mounted in buffer (pH 4.0). As previously stated, aniline blue and resorcinol blue are both specific for callose (O'Brien and McCully, 1981): following the aniline blue treatment, callose material fluoresces yellow when illuminated by ultra-violet light; after resorcinol blue treatment, callose stains cobalt blue when viewed in bright field illumination.

Fifty conidia were observed on each leaf disc and the following assessments made:

1. using the fungal development scale, the stage of development reached by each conidium;
2. for aniline blue staining, the number of small fluorescent areas at each infection site (Plate 4.4);

3. for aniline blue staining, the number of large fluorescent areas of each site (Plate 4.5);
4. for resorcinol blue staining, the number of areas stained cobalt blue at each site (Plate 5.1).

Only stained or fluorescing areas directly below appressoria or hyphae were recorded. After 18 hours, only germinated conidia were assessed.

Experiment 5.a(ii)

Plants of the seven cultivars used in the leaf surface studies were produced, sampled and inoculated (Section 2). Six days after inoculation, leaf discs were fixed for 24 hours in FPA, with the first 5 hours under vacuum (1 hour at 10 mg.Hg, 4 hours at 15 mg.Hg). After this, the leaf discs were dehydrated using a tertiary butyl alcohol (tBA) series, then infiltrated with paraffin wax (m.p. 52°C) as described by Purvis, Collier and Walls (1964):

Alcohol concentration	Period of immersion (hours)
70% tBA	72
85% "	72
90% "	72
95% "	72
Pure	72
tBA: paraffin liquid (1 : 1)	168
paraffin wax: "	168
paraffin wax (2 changes)	168

Following this, leaf discs embedded in paraffin wax were prepared and transverse sections (10 μm) were cut using a Cambridge rotary microtome. Haupt's adhesive (Gurr, 1965) was used to fix the sections to glass slides. Prior to staining, the sections were de-waxed by immersion

for 10 minutes in xylene and 10 minutes in ethanol. To detect callose, sections were stained overnight in aniline blue and examined using fluorescence microscopy.

Due to the variation in colony growth and, therefore, the number of attempted penetrations on resistant and susceptible hosts, transverse sections of each isolate/host combination were examined until 100 haustoria had been assessed. Assessments of the following were made:

1. number of small (<7 μm) and large (>7 μm) fluorescent areas (Plates 5.2 and 5.3);
2. number of haustoria and type of associated fluorescent area (Plates 5.4 and 5.5);
3. number of haustoria without associated fluorescence;
4. number of necrotic epidermal and/or palisade cells (Plates 5.6 and 5.7);
5. number of necrotic cells with haustoria (Plates 5.8 and 5.9).

Experiment 5.b(i)

In a parallel experiment to that described in Experiment 5.a(i), leaf discs were fixed and cleared in 95% ethanol and, following this treatment, three replicates were transferred to a saturated solution of dinitro phenylhydrazine (DNPH) in 15% acetic acid for 30 minutes, rinsed in distilled water for 4 minutes and subsequently immersed in Schiff's reagent for 150 minutes. Fungal structures were counterstained by mounting leaf discs in 0.01% trypan blue in lactophenol. The remaining three replicates were treated with 2% phloroglucinol in 95% ethanol for 60 minutes, placed in a drop of 3% hydrochloric acid on a slide and heated over a low flame until the veins turned reddish-purple (Sherwood and Vance, 1976). Fungal structures were counterstained by mounting leaf discs in 0.01% trypan blue in lactophenol.

→ *Plates 5. 1 – 5. 5*

PLATE 5.1: Callose deposit at site of penetration (resorcinol blue stain, x 100).

PLATE 5.2: Transverse section view of callose deposits (<7 µm diameter) at site of penetration (aniline blue stain, x 100).

PLATE 5.3: Transverse section view of callose deposits (>7 µm diameter) at site of penetration (aniline blue stain, x 100).

PLATE 5.4: Transverse section view of callose deposits (<7 µm diameter) associated with an haustorium (aniline blue stain, x 100).

PLATE 5.5: Transverse section view of callose deposits (>7 µm diameter) associated with an haustorium (aniline blue stain, x 100).

Plate 5.1

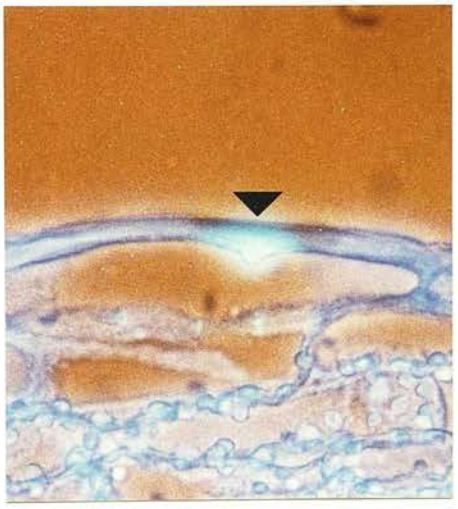


Plate 5.2

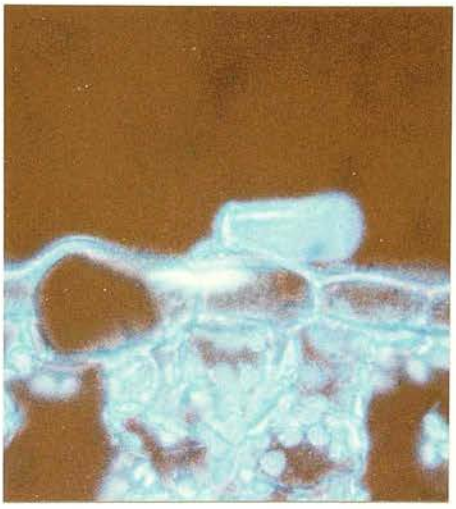


Plate 5.3

Plate 5.4

Plate 5.5

→ *Plates 5.6 – 5.10*

PLATE 5.6: Transverse section view of necrotic epidermal cell (x 100).

PLATE 5.7: Transverse section view of necrotic epidermal and necrotic palisade cells (x 100).

PLATE 5.8: Transverse section view of a degenerate haustorium within a necrotic epidermal cell (x 100).

PLATE 5.9: Transverse section view of an haustorium encapsulated with callose within a necrotic epidermal cell (aniline blue stain, x 100).

PLATE 5.10: Brown discolouration of leaf surface area around site of penetration (trypan blue stain, x 62.5).

Plate 5.6

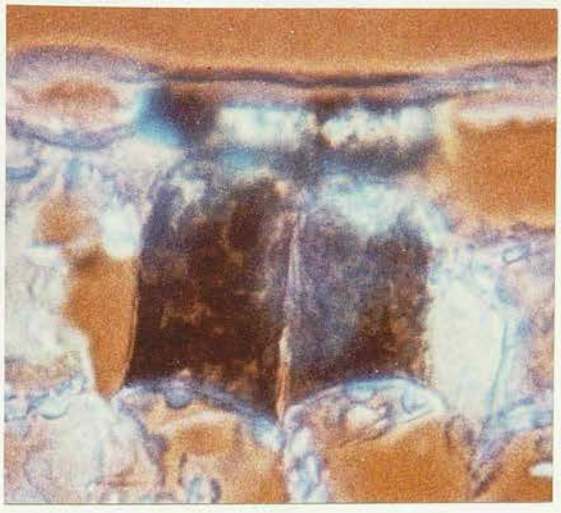


Plate 5.7

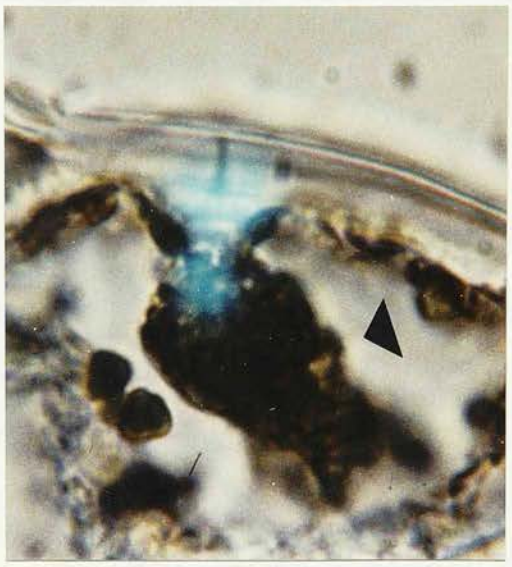


Plate 5.8

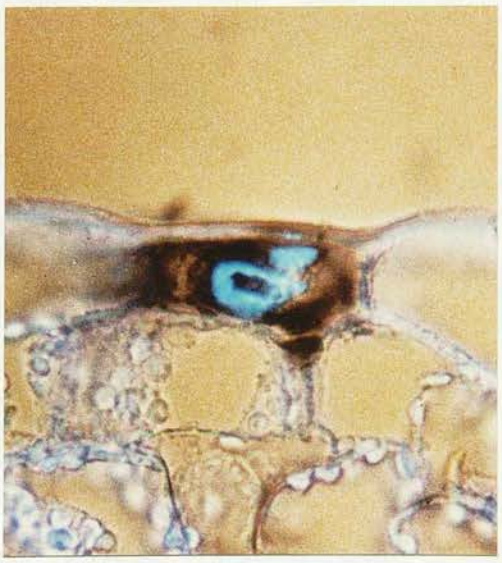


Plate 5.9

Plate 5.10

In tissues treated with Schiff's reagent, lignin aldehydes stain red (O'Brien and McCully, 1981) and, with phloroglucinol treated tissues, lignin stains red (Gurr, 1965).

This experiment was repeated twice, using formalin/propionic acid (FPA) fixative (90 cm^3 ethanol; 5 cm^3 propionic acid; 5 cm^3 formalin) and 70% ethanol to subsequently clear leaf discs. Six replicate leaf discs of each isolate/host combination were immersed in the FPA or 70% ethanol until they had cleared. Following clearing, three replicates were treated with DNPH and Schiff's reagent and the other three replicates treated with phloroglucinol. Leaf discs were examined under a light microscope and the presence of lignin and/or lignin aldehydes was recorded for 50 infection sites examined on each replicate leaf disc.

Experiment 5.b(ii)

An experiment was carried out with the two cultivars Doon Major (*B. napus*) and Vobra (*B. campestris*) used in the previous study, together with two other cultivars Lunet (*B. oleracea*) and Magres (*B. napus*). Test material was inoculated with isolates N2e, O8 and O4a. Inoculum was prepared by method A, leaf discs inoculated within the small settling tower and incubated for 7 and 14 days (Section 2).

Three replicate leaf discs of each isolate/host combination at both 7 and 14 days were treated, as previously described, with DNPH and Schiff's reagent and three replicates treated with phloroglucinol. All leaf discs were counterstained and mounted in 0.01% trypan blue in lactophenol and then examined under the light microscope. Twenty colonies on each replicate leaf disc were assessed.

Experiment 5.c

In the previous histochemical experiments a distinct brown discolouration of the host tissue in and around infection sites was noted (Plate 5.10). To investigate the possible presence of phenolic compounds associated with this discolouration, several different staining methods were used to examine further the response of four cultivars from the previous experiment, 144 hours after inoculation with isolate N2e. The stains were prepared as follows.

Diazotised sulphanilic reagent:

The diazotised salt was prepared (Smith, 1960) by adding dropwise a cold mixture of 225 cm^3 2N KOH, 50 g sulphanilic acid and 200 cm^3 10% $NaNO_2$, to a solution of 18N H_2SO_4 (80 cm^3) and water (40 cm^3) at 0°C. The precipitated p-sulphobenzenediazonium sulphate was filtered off, washed successively with ice-water, ethanol, ether, then air-dried at room temperature. The reagent was obtained by adding 0.4 g of the diazotised salt to 100 cm^3 2N NaOH. Several methods of staining with sulphanilic reagent were used. Firstly, after incubation, leaf discs were immersed directly into sulphanilic reagent and soaked overnight. Secondly, leaf discs were cleared in either 70% or 95% ethanol, and then immersed in sulphanilic reagent for 3 hours. Alternatively, leaf discs were mounted in the reagent and examined. Polyphenol compounds are stained orange-red (Mayama and Shishiyama, 1978).

Diazotised p-nitroaniline reagent:

Both the diazonium carbonate and the diazonium acetate were tested. They were prepared (Smith, 1960) using the following reagents:

Reagent	1	p-Nitraniline 0.3%, in 8% w/v HCl	(50 cm^3)
"	2	$NaNO_2$, 5% in water	(3 cm^3)
"	3	Na_2CO_3, 10% in water	(50 cm^3)
"	4	2M NaAC	(50 cm^3)

Diazonium carbonate was prepared by cooling reagents 1 and 2, mixing them and then adding cooled reagent 3. The diazonium acetate was prepared by substituting reagent 4 for Na_2CO_3. The staining procedures used were the same procedures as those used in the sulphanilic reagent tests. A yellow colour with the nitroaniline test indicates the presence of polyphenol compounds (Mayama and Shishiyama, 1978).

Toluidine blue:

A 0.1% toluidine blue solution in 0.1M phosphate buffer of pH 6.5 (Ride and Pearce, 1979) was prepared. Again, leaf discs were placed directly into the stain or leaf discs were cleared in 95% and 70% ethanol prior to immersion in toluidine blue overnight. Discs were removed from the stain, mounted in buffer (pH 6.5) and examined under a light microscope. A green reaction indicates the presence of polyphenolic material (O'Brien, Feder and McCully, 1964).

RESULTS

Experiment 5.a(i): *Leaf surface studies*

Positive staining reactions were obtained following both aniline blue and resorcinol blue treatment, indicating the presence of callose beneath sites of penetration. With the resorcinol blue test no differentiation of deposition sites into large or small was made but the results obtained were in agreement with those for the aniline blue study, and only the latter are reported here.

Small callose sites were first observed 8 hours after inoculation in association with conidia at the appressorium stage and large callose sites first observed after 18 hours at infection sites with appressoria (Table 5.1).

TABLE 5.1: Number of callose sites per colony in relation to time (mean of all hosts and isolates).

Callose sites	Hours after inoculation							SED ± (DF = 194)
	8	12	18	24	48	72	144	
<7 µm	0.04	0.09	0.19	0.20	0.45	0.84	0.91	0.051
>7 µm	0.00	0.00	0.02	0.12	0.14	0.25	0.32	0.021

More small callose sites per colony were found relative to large sites, but both tended to increase with time. The numbers of small callose sites differed significantly with host and isolate, but there were interactions between these factors and also between host, isolate and time. Significant differences in the number of large callose sites were found between host: the interactions between host and time and between host, time and isolate were also significant.

Small callose sites developed earlier and reached greater numbers on Doon Major than on any other host (Figure 5.1a). The frequency of small sites was generally intermediate on Barsica and Vobra and low on BC82, Achilles, Cluseed Early and RB25/8. Large callose sites also occurred earlier and in greater numbers on Doon Major compared with other hosts where numbers/colony were comparatively low, in particular on Cluseed Early and Achilles, on which no callose deposits were recorded until 48 hours after inoculation: with all other hosts, they were first observed at least 24 hours earlier (Figure 5.1b).

The extent of development of *E. cruciferarum* isolates varied between hosts, as illustrated in Figure 5.2a which gives the extent of development of colonies (mean of both isolates) on each host, by 144 hours after inoculation. Development was substantially greater on Doon Major and in decreasing order of Vobra, Barsica, BC82, RB25/8, Achilles and Cluseed Early. The total number (large and small) of callose sites/colony on each host increased with increasing extent of colony development (Figure 5.2b).

Isolate N2e developed to a greater extent on Barsica than colonies of isolate O4a, and produced greater numbers of callose sites. On other hosts both isolates tended to behave in a similar manner (Figure 5.3).

Experiment 5.a(ii): *Transverse section study*

From the examination of transverse sections, it was found that the deposition of callose occurred in two areas. It was found around the penetration hypha directly below the outer epidermal wall. Deposition in this area could be further categorised as small sites where the

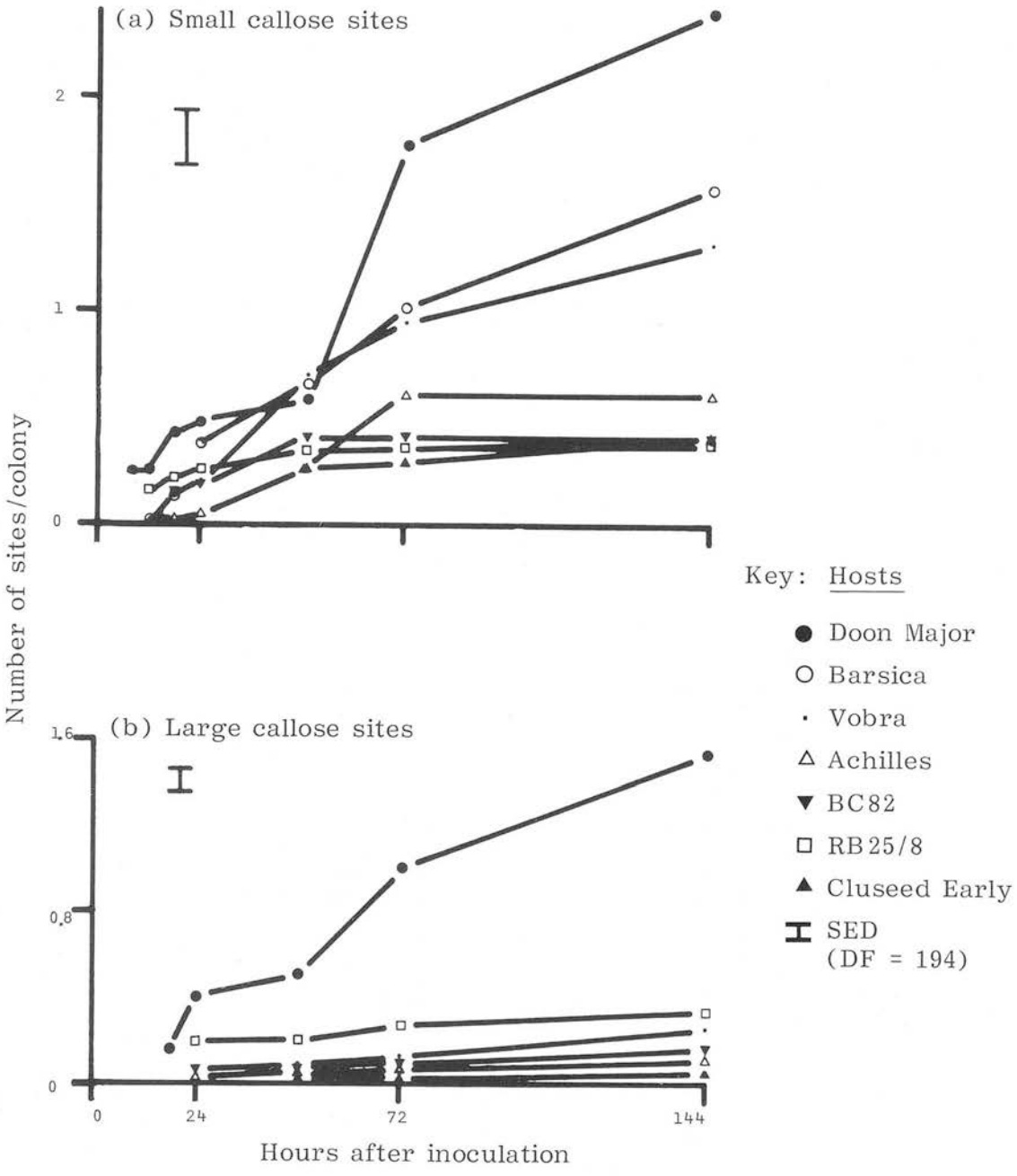


FIGURE 5.1: Influence of time on numbers of small (<7 μm) and large (>7 μm) callose deposits found with colonies of *E. cruciferarum* on different cruciferous hosts.

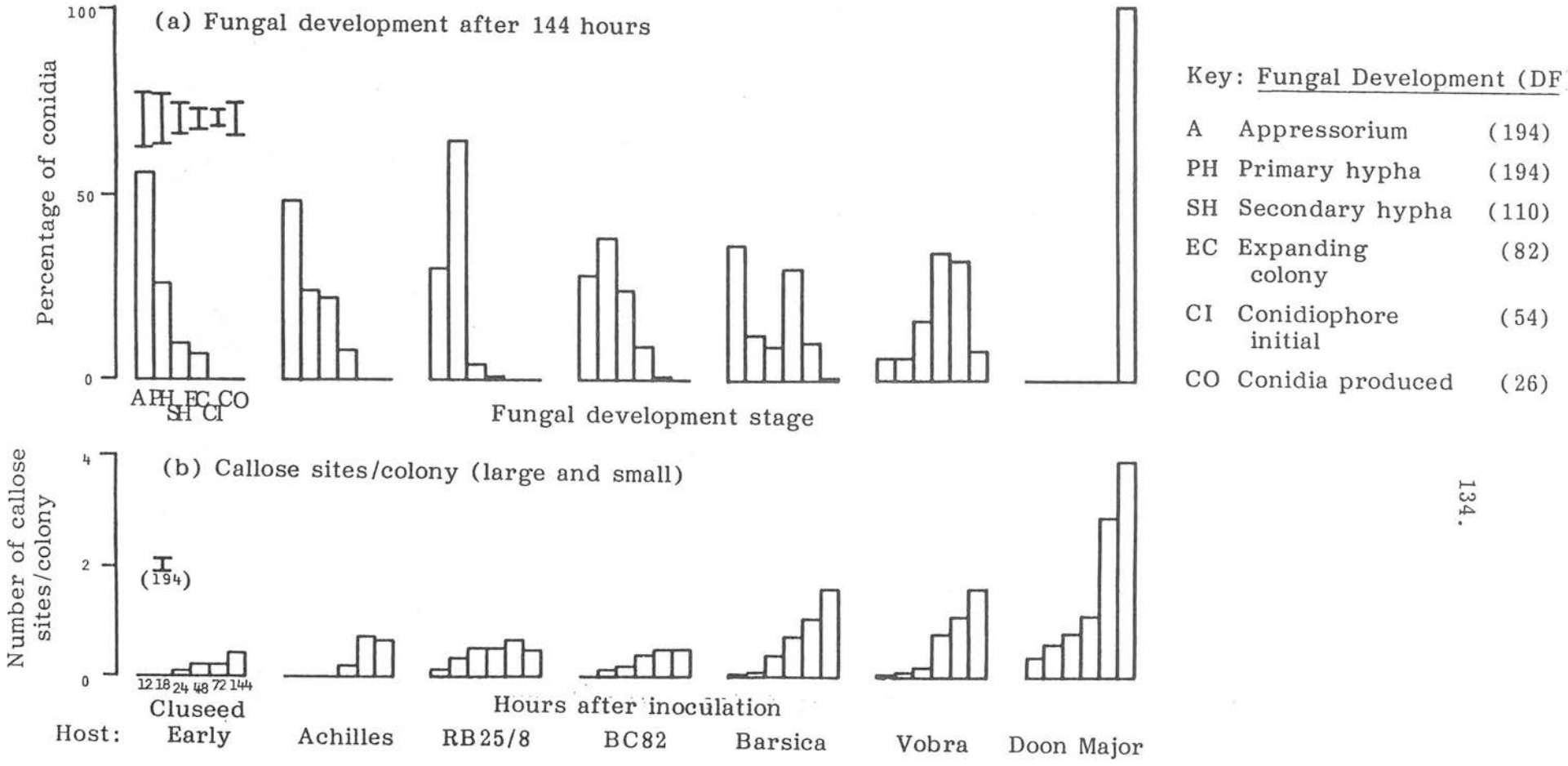
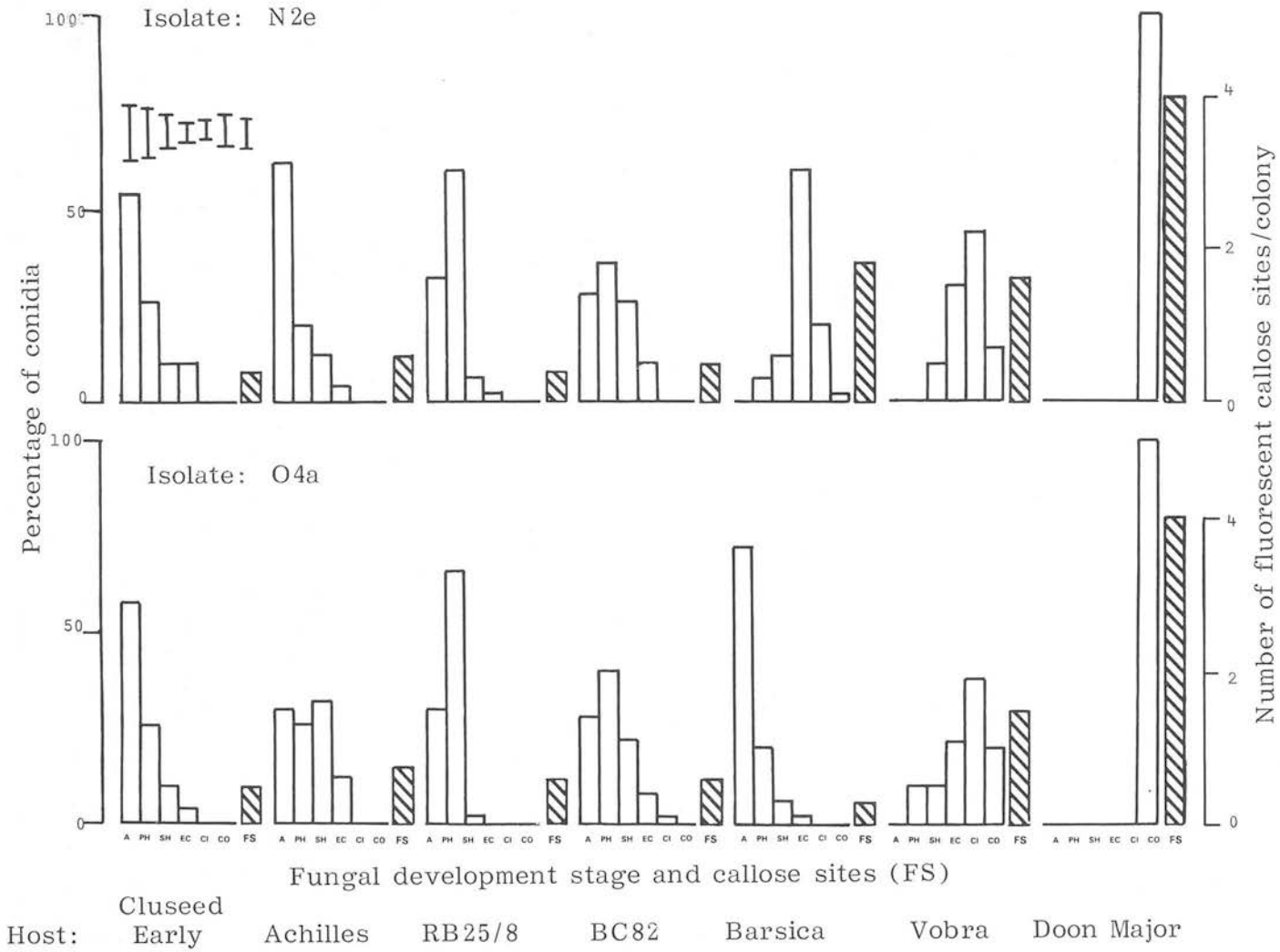


FIGURE 5.2: Extent of development of conidia of *E. cruciferarum* isolates 144 hours after inoculation and, the increase with time after inoculation (hours) of numbers of callose sites/colony.



Key:

Fungal development (DF)

A	Appressorium	(194)
PH	Primary hypha	(194)
SH	Secondary hypha	(110)
EC	Expanding colony	(82)
CI	Conidiophore initial	(54)
CO	Conidia produced	(26)
I	SED	
FS	Callose sites	(194)

FIGURE 5.3: Extent of development and number of callose sites/colony (FS) of two *E. cruciferarum* isolates on seven cruciferous hosts, 144 hours after inoculation.

callose deposits were less than 7 µm wide (Plate 5.4), or large sites where callose deposits were greater than 7 µm wide (Plate 5.5). Callose deposits were also found surrounding haustoria: the deposits engulfed a haustorium from the wall penetration area inwards (Plates 5.11 and 5.12).

Necrotic epidermal cells were found on all hosts and, in contrast to healthy cells which showed no discolouration, they had a dense black inner matrix; moreover the outer epidermal wall had collapsed (Plate 5.13). Haustoria found within necrotic cells were generally surrounded by callose (Plate 5.9). Necrotic cells were also found with callose deposits around the cell wall or within the cell, often with a granular appearance (Plate 5.14). Necrotic palisade cells were found, also with a dense black inner matrix (Plate 5.7).

From studies of transverse sections of cells with haustoria, five host responses to *E. cruciferarum* were identified.

1. no callose
2. callose deposits <7 µm
3. callose deposits >7 µm
4. encapsulation of haustoria by callose
5. cell necrosis.

The numbers of sites with haustoria with or without callose or necrosis and of sites with callose or necrosis without haustoria were considered to reflect the total number of penetration attempts and the proportions of the total in different categories are given in Table 5.2 and Table 5.3 Haustoria without callose were not found with RB25/8 and very few occurred with Achilles and isolate O4a on BC82: on other cultivars the percentages ranged from 4 to 26% of all penetration attempts for different isolate/host pairings, the highest frequency occurring with O4a on Doon

→ *Plate 5. 11 – 5. 14*

PLATE 5.11: Transverse section view of an haustorium of *E. cruciferarum* partly encapsulated with callose (aniline blue stain, x 100).

PLATE 5.12: Transverse section view of an haustorium of *E. cruciferarum* encapsulated with callose (aniline blue stain, x 250).

PLATE 5.13: Transverse section view of a collapsed necrotic epidermal cell (x 100).

PLATE 5.14: Transverse section view of a necrotic epidermal cell with granular deposits of callose (aniline blue stain, x 100).

Plate 5.11

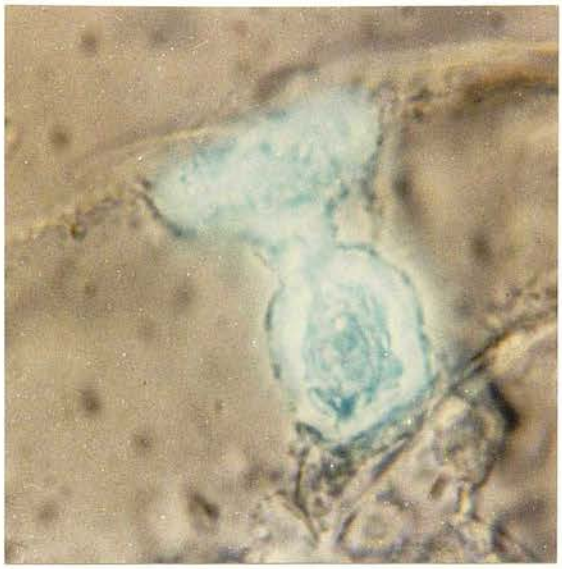


Plate 5.12

Plate 5.13

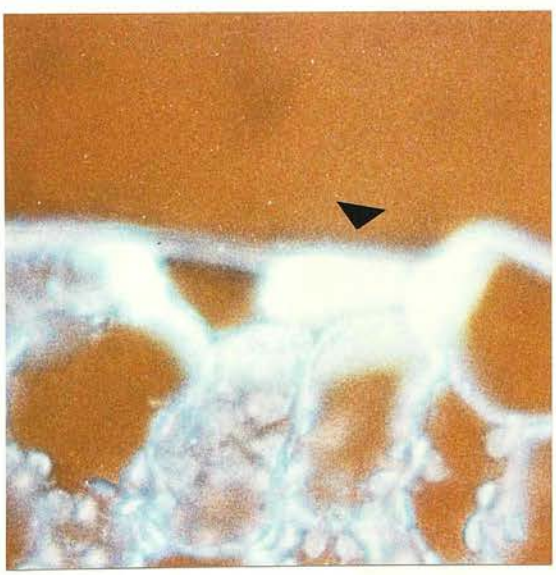


Plate 5.14

TABLE 5.2: Percentage of penetration attempts with haustoria and haustoria associated with callose and necrotic cells on seven hosts at 120 hours after inoculation with two *E. cruciferarum* isolates.

% Penetration attempts with haustoria	Isolate	Host: Doon Major	Barsica	Vobra	BC82	Achilles	Cluseed Early	RB25/8	Mean
No callose deposit	N2e	8	6	12	6	1	5	0	5.5
	O4a	26	9	4	1	2	7	0	7
	Mean	*17*	*7.5*	*8*	*3.5*	*1.5*	*6*	*0*	*6*
Small callose deposit	N2e	22	18	42	13	2	13	0	16
	O4a	38	15	10	3	2	8	3	11
	Mean	*30*	*16.5*	*26*	*8*	*2*	*10.5*	*1.5*	*13.5*
Large callose deposit	N2e	30	15	24	11	1	3	0	12
	O4a	20	14	10	7	4	7	0	9
	Mean	*25*	*14.5*	*17*	*9*	*2.5*	*5*	*0*	*10.5*
Encapsulated with callose	N2e	3	12	2	10	6	5	14	7.5
	O4a	2	8	10	8	5	7	19	8
	Mean	*2.5*	*10*	*6*	*9*	*5.5*	*6*	*16.5*	*7.7*
Necrotic cell	N2e	4	12	0	4	13	6	10	7
	O4a	0	5	0	5	11	13	3	5
	Mean	*2*	*8.5*	*0*	*4.5*	*12*	*9.5*	*6.5*	*6*
Total % with haustoria	N2e	67	63	80	44	23	32	24	48
	O4a	86	51	34	24	24	42	25	40
	Mean	*76.5*	*57*	*57*	*34*	*23.5*	*37*	*24.5*	*44*

TABLE 5.3: Percentage of penetration attempts without haustoria, but with associated callose or necrotic cells on seven hosts at 120 hours after inoculation with two *E. cruciferarum* isolates.

% Penetration attempts without haustoria but associated with	Isolate	Host: Doon Major	Barsica	Vobra	BC82	Achilles	Cluseed Early	RB25/8	Mean
Small callose deposits	N2e	18	14	15	3	11	14	12	12.5
	O4a	3	15	34	24	11	4	9	14
	Mean	*10.5*	*14.5*	*24.5*	*13.5*	*11*	*9*	*10.5*	*13*
Large callose deposits	N2e	10	14	3	15	19	17	27	15
	O4a	11	21	23	26	23	7	21	19
	Mean	*10.5*	*17.5*	*13*	*20.5*	*21*	*12*	*24*	*17*
Necrotic epidermal cells	N2e	5	8	1	38	41	37	36	24
	O4a	0	14	10	23	39	47	46	26
	Mean	*2.5*	*12*	*5.5*	*30.5*	*40*	*42*	*41*	*25*
Necrotic palisade cells	N2e	0	0	0	0	5	0	0	0.7
	O4a	0	0	0	3	4	0	0	1.0
	Mean	*0*	*0*	*0*	*1.5*	*4.5*	*0*	*0*	*0.85*
Total % without haustoria	N2e	33	36	19	56	76	68	75	52
	O4a	14	50	67	76	77	58	76	60
	Mean	*23.5*	*43*	*43*	*66*	*76.5*	*63*	*75.5*	*56*

Major (Table 5.2). Haustoria with small or large callose deposits were also absent from RB25/8 except for some occurrence of small callose deposits with isolate O4a: callose sites associated with haustoria tended to be relatively infrequent with Achilles: the highest numbers were found with Doon Major and Vobra, particularly in the case of small sites from N2e on Vobra (42%) and large sites from N2e on Doon Major (30%). The proportion of encapsulated haustoria was very low with Doon Major and highest with RB25/8 (16.5% averaged for the two isolates). Of the 100 haustoria assessed on RB25/8, 68 were encapsulated (16.5% of all penetration attempts). Necrotic cells with haustoria were absent on Vobra and very low with Doon Major, while the highest percentage (12% on average) was found with Achilles. Of the 100 haustoria assessed on Achilles 50 were associated with host cell necrosis, whereas on Doon Major, only three were found with necrotic cells.

There was a wide range of percentages of penetration sites without haustoria found on different hosts (Table 5.3). The lowest numbers were found on Doon Major (14-33%), whereas on Achilles and RB25/8 at least 75% were in this category. The mean percentage of unsuccessful penetration attempts with necrotic cells without haustoria found on each host varied considerably: on Doon Major, Vobra and Barsica relatively low numbers were found; on BC82 the percentage was intermediate (23-38) but on Achilles, Cluseed Early and RB25/8 about 40% of penetration (36-47%) with necrotic cells without haustoria were found (Table 5.3). On Achilles and BC82 a small percentage of sites with necrotic palisade cells were observed. Variation between isolates on each host occurred with all categories assessed, but no clear patterns emerged (Table 5.2 and 5.3).

Experiment 5.b: *Lignin and lignin aldehyde formation*

(i) On uninoculated controls and inoculated leaf discs, xylem vessels were stained red after treatment with Schiff's reagent and phloroglucinol. However, no staining indicative of lignin aldehydes or lignin associated with infection sites was found on any cultivar during the period of assessment. This negative result occurred regardless of whether the leaf discs had been fixed in 95% ethanol, 70% ethanol or FPA.

(ii) As in the previous study, after phloroglucinol treatment no staining reaction for lignin was found below fungal structures on any cultivar, either at 7 or 14 days after inoculation, although on both uninoculated controls and inoculated leaf discs xylem vessels were stained red after treatment with phloroglucinol. However, on inoculated leaf discs, following treatment with DNPH and Schiff's reagent, a positive staining reaction was found below fungal structures 14 days after inoculation.

Thus lignin aldehydes were not evident during the first 7 days after inoculation, but were formed at penetration sites between 7 and 14 days after inoculation on all cultivars. The differentiation of callose staining into small (<7 μm diameter) and large (>7 μm diameter) was, also, applied to the assessments of the lignin aldehyde staining (Plates 5.15, 5.16). The number of both small and large areas stained with Schiff's reagent varied significantly with host and with isolate and their interaction (Table 5.4). The greatest number of small stained areas was found on Magres; numbers on Lunet were intermediate while on Doon Major and Vobra a relatively low frequency was observed. On the other hand, the numbers of large stained areas were greater on Doon Major than on any other host, although the mean number was relatively low.

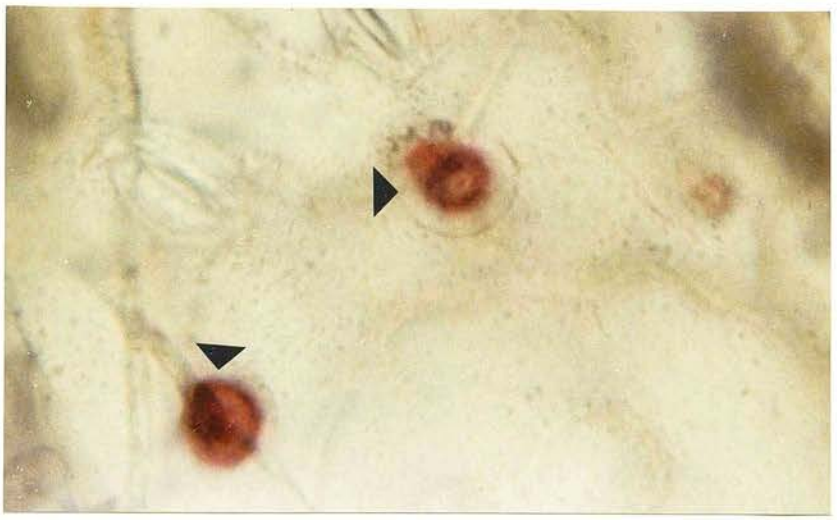


PLATE 5.15: Lignin aldehyde deposits (<7 µm diameter) at site of penetration (stained with Schiff's reagent, x 100).

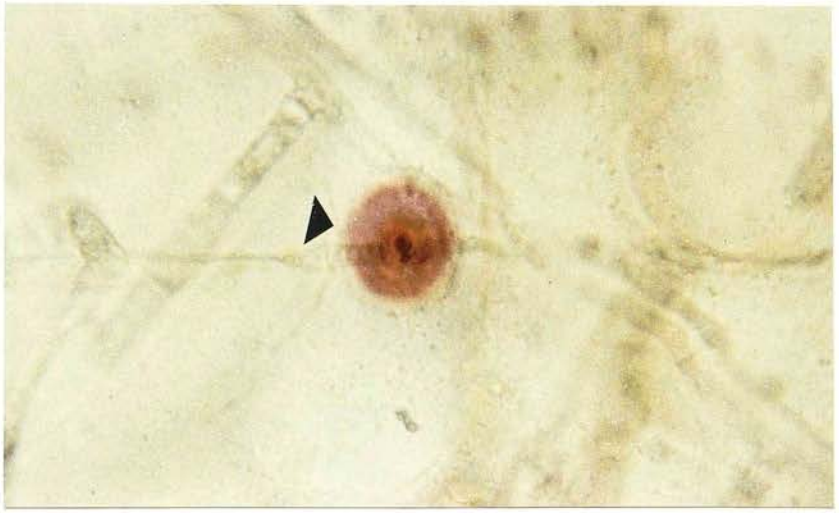


PLATE 5.16: Lignin aldehyde deposits (>7 µm diameter) at site of penetration (stained with Schiff's reagent, x 100).

TABLE 5.4: Mean number per colony of small and large areas of lignin aldehyde found beneath colonies of three isolates on each of four hosts at 14 days after inoculation.

Areas of lignin aldehydes	Isolates	Hosts					SED ± (DF = 46)	
		Doon Major	Vobra	Lunet	Magres	Mean		
Small	O8	1.2	0.7	2.1	2.3	1.6	Isolate	0.05
	N2e	1.1	2.0	0.9	2.0	1.5	Host	0.06
	O4a	1.0	0.2	1.0	0.6	0.7	Interaction	0.14
	Mean	*1.1*	*1.0*	*1.3*	*1.6*	-		
Large	O8	0.7	0	0.1	0	0.2	Isolate	0.02
	N2e	0.8	0.5	0.2	0.1	0.4	Host	0.02
	O4a	0.8	0	0.1	0.1	0.2	Interaction	0.15
	Mean	*0.7*	*0.2*	*0.1*	*0.1*			

The numbers per colony of both large and small areas on Doon Major were similar with each isolate. The three isolates produced the different numbers of small stained areas beneath colonies on Vobra, Lunet and Magres. On Vobra, isolate N2e gave more stained areas/colony than isolate O8 and O4a, which gave the lowest. On Lunet, however, the highest frequency of small sites was found with colonies of isolate O8: numbers were intermediate with both N2e and O4a. The frequency of small sites on Magres was greater beneath colonies of O8 and N2e than beneath colonies of O4a. In general, isolates O8 and N2e were associated with more areas with lignin aldehydes than isolate O4a. The mean number of large areas/colony was only 0.2 or less on Lunet and Magres for the three isolates but, on Vobra N2e showed 0.5/colony while the other isolates showed none.

Experiment 5.c: *Phenolic compound formation*

On no occasion was a positive staining reaction obtained following application of any of the techniques described, i.e. diazotised sulphanilic reagent, diazotised p-nitroaniline reagent or toluidine blue, on any cultivar 144 hours after inoculation.

DISCUSSION

The presence of callose at sites of infection was confirmed in staining tests using resorcinol blue or aniline blue. This deposition of callose was found, in general, to be localised around penetration hyphae and directly above haustoria (Plate 5.17), and conformed with earlier observations (Akai, 1959; Bracker and Littlefield, 1973) that deposits form between the inner cell wall and plasmalemma, resulting in a thickening of the cell wall below the infection site. If these appositions continue they may become dome-shaped (i.e. papillae) or elongate (i.e. discs) (McKeen, Smith and Bhattacharya, 1969; Aist, 1976). Sherwood and Vance (1976) distinguished between papillae and discs, but they found callose to be present in both.

PLATE 5.17: Transverse section of callose deposition above haustorium of *E. cruciferarum*.

The role of callose deposition by host cells in response to infection continues to be the subject of much debate, having been implicated both in host resistance (Sherwood and Vance, 1976) and as a generalised response to injury (Eschrich and Currier, 1964). In Experiment 5.a and in previous experiments (4.a, 4.b, 4.c), the number of callose sites increased with increasing host susceptibility. Numbers of both small and large callose sites were greatest with colonies on Doon Major and numbers increased per colony with time from 18 hours after inoculation. From the assessment of transverse sections (Experiment 5.a(ii)), 75% of haustoria in epidermal cells of Doon Major were associated with callose. In this study the distinction between small and large sites appears to correspond with previously described papillae and discs. The results again showed that the formation of both small and large callose appositions occurred in response to penetration and not as a generalised host resistance reaction: thus, callose deposits were evident in both susceptible and resistant hosts, in agreement with several earlier workers (Eschrich and Currier, 1964; Mayama and Shishiyama, 1976, 1978).

In the previous experimental section, germination and appressorial formation occurred synchronously on different hosts, while penetration has been shown to occur simultaneously on resistant and susceptible hosts (Bushnell and Berquist, 1975; Maclean and Tommerup, 1979). Here and in earlier experiments (4.b, 4.c), callose deposition occurred earlier on Doon Major than on less susceptible hosts and subsequent development beyond the appressorial stage was also more rapid on Doon Major: immature haustoria were observed beneath appressoria at 18 hours only on Doon Major, and not until 48 hours after inoculation on all other hosts. This implies that during primary infection stages

penetration is retarded on the resistant hosts, such that a response, i.e. callose deposition, by these resistant hosts is not elicited as early as with Doon Major. In consequence, callose deposition occurs later, haustorial formation is later and colony development retarded.

Although callose deposition in the hosts tested was considered to occur in response to penetration, with one exception the ratio of small to large sites increased with increasing susceptibility, and suggests that increasing local callose deposition may play a role in host resistance: Koga *et al.* (1980) found a correlation between increasing callose deposition and resistance. In some cases in the present study the deposition of callose was found to continue at the initial apposition, progressively encapsulating haustoria which had formed (Plates 5.11, 5.12). This type of callose formation has not previously been reported. In Table 5.2, it is seen that the percentage of haustoria encapsulated with callose was higher on more resistant hosts. On the resistant host RB25/8, 68% of haustoria were encapsulated by callose. Biotrophic fungi extract nutrients from the host cell across the interface between the haustorial membrane and host plasmalemma. Callose has been found to restrict the passage of molecules between tissues (Heslop-Harrison, 1966). Thus, haustorial encapsulation between the two membranes would have an adverse effect upon the nutrition of the pathogen. The gradual build up of callose occurs from the inner cell wall and progressively develops around the penetration hypha to the distal end of the haustorium (Plates 5.11, 5.12). This increase in callose with time is also consistent with observations of Koga *et al.* (1980). From the assessment of transverse section studies and in accordance with earlier workers (Sherwood and Vance, 1976), the ability of a host to encapsulate haustoria with callose may be considered a defence factor to infection by *E. cruciferarum*.

In addition to the small and large callose depositions found in host cells at sites of fungal penetration, other responses were observed: callose deposits were identified in lateral walls (Plate 5.18) as forming granular areas around the site of penetration (Plate 5.19), particularly in necrotic cells (Plate 5.20), and as haloes around the site of penetration (Plate 5.21). These responses were, with one exception, found only on the more resistant hosts and are, therefore, likely to play a role in host resistance, consistent with observations by Mayama and Shishiyama (1976) and Sherwood and Vance (1976).

In the previous experimental section, it was concluded that cell necrosis plays a role in resistance of cruciferous hosts to *E. cruciferarum*. In Experiment 5.a(ii), an inverse relationship was found between percentage of successful penetration attempts and percentage of cell necrosis (Table 5.5). On Doon Major and Vobra, only about 4% and 5% respectively, of all penetration attempts initiated a necrotic response, whereas with Barsica and BC82 the percentages were 20 and 37, and with the other hosts over 45%.

In addition, assessments of transverse sections (Experiment 5.a) showed that, with the more resistant hosts (Cluseed Early, BC82, RB25/8, Achilles), the frequency of successful penetration attempts declined while the percentage of haustoria associated with cell necrosis or encapsulated with callose increased with increasing host resistance (Table 5.2).

In Experiment 5.b, lignified materials in appositions and lateral walls in response to infection were detected. In agreement with Sherwood and Vance (1976), the central core of appositions were often more intensely stained than the outer area (Plate 5.22). Necrotic epidermal cells were also identified due to their intense staining reaction with Schiff's reagent (Plate 5.23). The negative results after treatment with phloroglucinol

→ *Plates 5. 18 – 5. 21*

PLATE 5.18: Callose deposition in epidermal cell walls (aniline blue stain, x 100).

PLATE 5.19: Granular callose deposits around site of penetration (aniline blue stain, x 100).

PLATE 5.20: Granular callose deposits within necrotic epidermal cell (aniline blue stain, x 100).

PLATE 5.21: Callose halo around site of penetration (aniline blue stain, x 100).

Plate 5.18

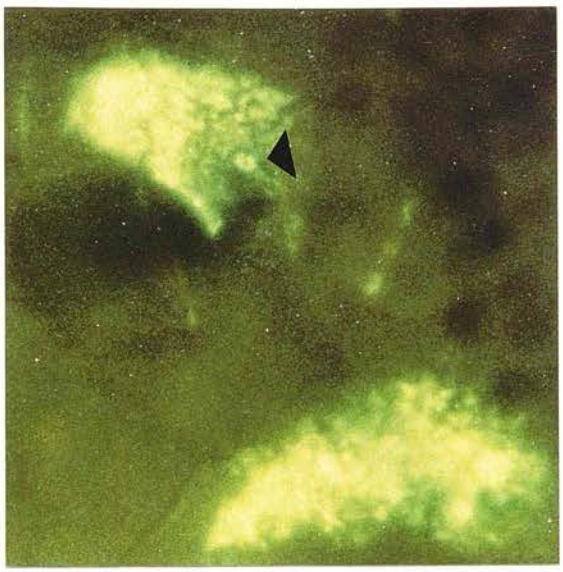


Plate 5.19

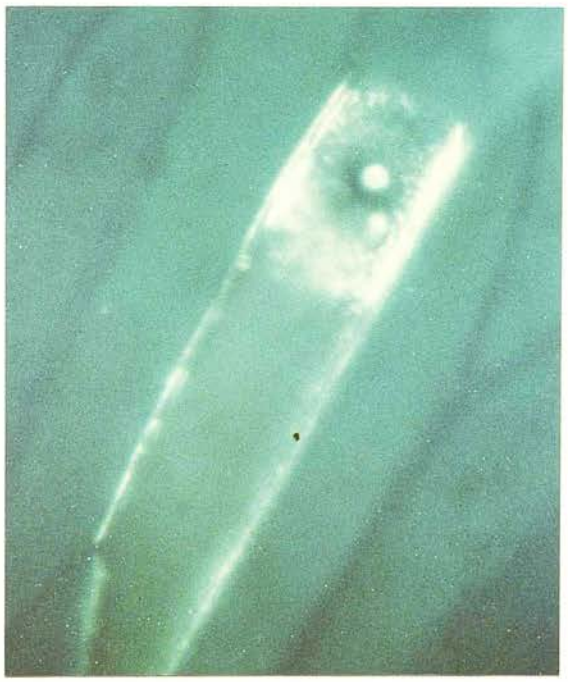


Plate 5.20

Plate 5.21

TABLE 5.5: Influence of host on the frequency of haustorial formation and, host response to penetration by isolates of *E. cruciferarum* 120 hours after inoculation.

	Hosts						
	Doon Major	Barsica	Vobra	BC82	Achilles	Cluseed Early	RB25/8
Total* number of penetration attempts observed	135	180	216	325	427	276	408
% Successful (with haustoria)	76.5	57	57	34	23.5	37	24.5
% Unsuccessful (without haustoria)	23.5	43	43	66	76.5	63	75.5
% Penetrations with callose	78	72.5	86.5	59.5	42	42	52.5
% Penetrations with necrosis	4.5	20.5	5.5	37	56.5	51.5	47.5

*Larger number of penetration attempts on the more resistant hosts reflects the number of sites necessary to assess in order to obtain 100 haustorial assessments with each isolate/host combination.

indicate that no cinnamaldehyde end groups are present within the lignified deposits identified by Schiff's reagent.

It has been demonstrated that the formation of lignified appositions between the cell wall and plasmalemma, as well as lignification of cell walls, are important in the resistance of graminaceous hosts to several fungi (Ride, 1975; Vance and Sherwood, 1977; Sherwood and Vance, 1980). In Section 4 the number of haustoria/colony 14 days after inoculation ranged between 6 to 8 on each of the four hosts tested. The numbers of lignified appositions/colony found at this time on each host was much lower, i.e. approximately 1 to 2 per colony. As greater numbers of lignified appositions occurred with colonies on the susceptible Doon Major, the possible role of lignified material in host resistance is unconfirmed.

The rapid formation of lignified materials in papillae as a mechanism of resistance has been suggested (Ride, 1975; 1978). In the present studies, lignin aldehydes were not detected until 14 days after inoculation by which time colony development was extensive on Doon Major. In addition, at each site where lignin aldehydes were detected, an haustorium was found (Plates 5.24 and 5.25). These observations do not accord with those of Wardrop (1971) who concluded that lignification of papillae acts as a mechanical barrier to penetration. It has been suggested that the apparent time of initiation of lignification is dependent upon the detection method employed (Ride and Pearce, 1979). Their results using the traditional histochemical tests, i.e. phloroglucinol or chlorine-sulphate, indicated that lignification either did not occur or probably occurred too late to be of any significance. In contrast, toluidine blue and autofluorescence detected lignification at an early stage. However, in each of the histochemical tests used to identify lignin materials, the tests detected these constituents within

→ *Plates 5. 22 – 5. 25*

PLATE 5.22: Lignin aldehyde deposits with a more dense central core at site of penetration (stained with Schiff's reagent, x 100).

PLATE 5.23: Necrotic epidermal cell (stained with Schiff's reagent, x 100).

PLATE 5.24: Lignin aldehyde deposit above an haustorium of *E. cruciferarum* (stained with Schiff's reagent, x 100).

PLATE 5.25: Lignin aldehyde deposit above an haustorium of *E. cruciferarum* (stained with Schiff's reagent, x 100).

Plate 5.22

Plate 5.23

Plate 5.24

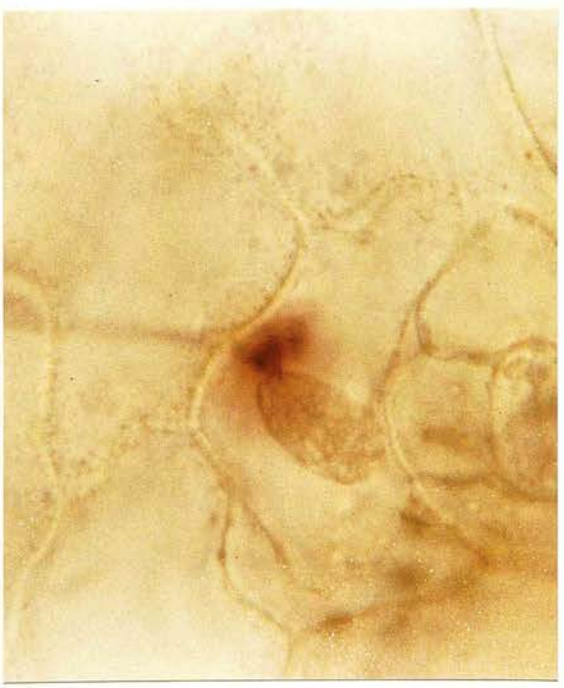


Plate 5.25

xylem tissues at each assessment time. Therefore, had any lignified materials been present in appositions or lateral walls prior to the 14 day assessment, they also would have been detected by the histochemical tests employed.

The delay in lignification, the relatively low frequency of appositions of these constituents, the association of lignified appositions with haustoria, and the greater numbers of appositions with colonies of a compatible isolate/host combination provide no evidence that a lignification response is a factor of the resistance of the hosts tested. As previously shown (Section 4), at later stages of infection degenerate haustoria and host cells are found in greater numbers in compatible than incompatible isolate/host combinations associated with the exhaustive demands of the compatible fungus. The observations by Maule (1977), that lignification can occur in degenerating cytoplasm and cell walls, appears a more likely explanation for the presence of lignified materials found in this study.

Although phenolic substances have often been implicated in the resistance response of plant tissues to infection, Experiment 5.c failed to demonstrate their presence in association with *E. cruciferarum* infection of susceptible or resistant tissues.

From these experiments, in agreement with previous experiments, callose deposition occurs in response to penetration with all hosts: with susceptible hosts it does not appear to impair fungal development but, with the more resistant hosts, callose deposition may continue and restrict penetration and haustorial development and/or immobilise haustoria by encapsulation with callose. In addition, fungal development may be further restricted by early cell necrosis.

6. INFLUENCE OF INTRA-CULTIVAR VARIATION AND PREDISPOSITION FACTORS ON HOST SUSCEPTIBILITY

INTRODUCTION

The previous experimental studies have been concerned largely with the expression of the interaction between the genetical systems of host and pathogen with cruciferous plants and *E. cruciferarum*, using cultivars or lines of species from the family Cruciferae as the host units and single spore isolates of the fungus from different sources as the pathogen unit. However, as a result of the outbreeding habit which characterises most of the Cruciferae, variation between plants within cultivars may occur. Variations in host susceptibility have been associated with other factors: for example, Jones and Hayes (1971) demonstrated adult plant resistance in oat cultivars to *E. graminis* f. sp. *avenae* with both leaf and plant maturity affecting susceptibility. Environmental factors which may influence the predisposition of plants to infection include temperature, water availability, nutrient status and light (Yarwood, 1957; Schoeneweiss, 1975). In addition, the composition and structure of cuticular surfaces have been demonstrated to play a role in resistance (Brown, 1922; Martin, 1964).

The experiments in this section were carried out to examine the variability in host susceptibility to *E. cruciferarum* which may be associated with intra-cultivar variation or host predisposition factors. The effects of (a) intra-cultivar variation, (b) plant age, (c) leaf age, (d) pre-inoculation temperature, (e) fertiliser treatment, (f) leaf type and (g) leaf washing treatment on host-susceptibility to mildew infection were investigated.

MATERIALS AND METHODS

In each of the experiments of this section, unless otherwise stated, plant material was prepared by method B (Section 2). In Experiments 6.a, .b, .c and .g leaf discs were inoculated in the large settling tower, whereas the small tower was employed in Experiments 6.d, .e and .f. The 0-5 Disease assessment scale was used to assess infection 6 days after incubation under standard conditions. Isolates N2e and O4a were used in all experiments, while additional isolates were also used in Experiments 6.b, .d, .f and .g.

Experiment 6.a

Five seed collections of each of eight *Brassica* cultivars were obtained from different sources (Table 6.1), the availability of seed collections determining which cultivars were used. Two groups of plants were produced, each comprising six replicates of each of the five seed samples representing each of eight cultivars. Test material from the first group was inoculated 7 weeks after sowing, and from the second group 13 weeks after sowing.

Experiment 6.b

Cultivars Doon Major and Magres (*B. napus*), Lunet and Decema (*B. oleracea*), and Vobra (*B. campestris*) were each sown at weekly intervals over 5 weeks, followed by two sowings at fortnightly intervals. Test material was prepared, taking discs from the first formed leaf of each plant, and inoculated 5 weeks after the final sowing giving an age range of plants of 5, 7, 9, 10, 11, 12 and 13 weeks. On the youngest plants, the first formed leaf was not large enough to yield

TABLE 6.1: Source of seed collections of eight *Brassica* cultivars.

Cultivar	Crop type	Species	Seed collection source*
Doon Major	swede	*B. napus*	1, 2, 3, 4, 5
Rape Kale	rape		2, 3, 6, 7, 8
Ruta Otofte	swede		1, 2, 3, 7, 8
Jet Neuf	oilseed rape		3, 9**a,b,c, 10
Bruce	yellow turnip	*B. campestris*	2, 4, 5, 6, 7
Wallace	yellow turnip		2, 4, 5, 7, 11
Vobra	stubble turnip		2, 3, 5, 6, 7
Nevin	forage rape		1, 2, 5, 8, 12

* 1. Scottish Crop Research Institute, Pentlandfield.

2. East of Scotland College of Agriculture, Advisory and Development Department, Edinburgh.

3. East of Scotland College of Agriculture, Crop Production Department, Edinburgh.

4. Charles Sharpe & Co. Ltd.

5. Sinclair McGill Ltd.

6. West of Scotland College of Agriculture, Advisory and Development Department, Ayr.

7. Asmer Wholesale Ltd.

8. Edwin Tucker & Sons Ltd.

9. National Institute of Agricultural Botany (**a, b and c from different seed batches).

10. K.P.R. Prasanna, Edinburgh.

11. Barclay Ross & Hutchison.

12. National Seed Development Organisation.

five standard size discs and so a smaller (No. 7) cork borer was used to cut discs. Inoculum was prepared by method A using isolates N2e, O4a, N2d, N3a and O8. Each host/isolate combination was replicated four times for every sowing date.

Experiment 6.c

Plants of eight cultivars, four of *B. napus* (Doon Major, Ruta Otofte, Jet Neuf, Rape Kale) and four of *B. campestris* (Wallace, Nevin, Bruce, Vobra), were produced in a glasshouse. Leaf discs from the eight youngest expanded leaves were prepared and inoculated 13 weeks after sowing. Each host/isolate/leaf combination was replicated three times.

Experiment 6.d

The cultivars Lunet (*B. oleracea*) and Magres, Vobra and Doon Major (*B. napus*) were sown together on four different dates, each 3 weeks apart. To compare the effect of different temperatures during plant growth on host resistance, sets of plants of all cultivars at every sowing date were grown in a controlled environment cabinet at cool temperatures (mean of 9°C; range 6.5 to 12.5°C) and further sets at warm temperatures (mean of 22°C; range 16.5 to 26°C). All plants received 16 hours light (250 Lux) per day. Test material was inoculated with isolates N2e, O4a and O8 4 weeks after the final sowing, giving a cultivar age range of 4, 7, 10 and 13 weeks. A No. 7 cork borer was used to remove discs from test material of the youngest plants. Each host/isolate combination was replicated six times at each sowing date.

Experiment 6.e

Twelve plants of five *Brassica* cultivars, Doon Major and Magres (*B. napus*), Vobra (*B. campestris*), Lunet and Decema (*B. oleracea*) were allocated to two groups, each to represent six replicates of each of the five cultivars. Plants in one group were given Sangral liquid fertiliser, and plants in the other group given Solufeed (Section 2). Test material was inoculated 13 weeks after sowing. After removal of the leaf discs required for infection studies, the remaining leaf material of each cultivar in each group was dried overnight at 85°C. An analysis of the manganese concentration (ppm DM*) of each cultivar from each group was then carried out by atomic absorption spectrophotometry.

Experiment 6.f

Seedlings of breeding lines A4207, A8022 and A8024 of *B. oleracea* var. *gemmifera*, received from the Scottish Crop Research Institute, were classified 2 to 3 weeks after sowing into normal or glossy (Wills, pers. comm.) and selections of each type along with Doon Major seedlings were then transplanted individually into pots and grown on (Section 2). Six replicate plants of Doon Major and of each leaf type of each line were prepared and inoculated with isolates N2e, O4a and N4a 7 weeks after sowing, and a further six replicates at 13 weeks.

Experiment 6.g

Plants of cultivars Doon Major, Barsica (*B. napus*), Vobra, Achilles (*B. oleracea*) and line BC82 (*B. carinata*) were grown to produce leaf material for testing. Ten treatments (Table 6.2) were applied to the leaf discs, involving rinsing for varying periods of time

*Dry matter

in 70% ethanol (+ 0.15% Tween 90) and, in some treatments, a further 15 minute rinse in sterile distilled water. With both alcohol and water rinses, leaf discs were placed into beakers and gently stirred throughout the period of immersion. After treatment the leaf discs were lightly blotted with sterile filter paper and air dried prior to transferring to agar plates and inoculating with *E. cruciferarum* isolates. Seven week old plants were inoculated with isolates N4a and O4a and, 13 week old plants inoculated with isolates N2e and O4a.

TABLE 6.2: Washing treatments applied to leaf discs.

Treatment	Immersion period in 70% ethanol (+0.5% Tween 90)	Immersion period in sterile distilled water for 15 minutes
1	-	-
2	-	+
3	10 seconds	+
4	30 "	+
5	60 "	+
6	100 "	+
7	150 "	+
8	10 "	-
9	60 "	-
10	150 "	-

RESULTS

Experiment 6.a: *Mildew development on leaf tissues from plants grown from different seed collections of Brassica cultivars*

The analysis of variance of the results of disease assessments for plants inoculated at 7 weeks showed significant cultivar effects and significant interactions between cultivar and source, and cultivar and isolate. In the parallel studies on 13 week old plants cultivar and isolate showed significant effects, but not source, and there were no significant interactions between any of the factors.

When inoculated at 7 weeks Doon Major and Nevin showed the highest disease scores, Wallace, Bruce and Ruta Otofte were intermediate, while Vobra, Jet Neuf and Rape Kale showed the least disease (Table 6.3). Disease assessment scores were generally much higher when leaf discs of 13 week old plants were used for inoculation. Doon Major and Nevin again showed the highest disease scores, and Bruce and Wallace gave disease ratings above the remaining four cultivars, which still gave higher scores than any of the cultivars inoculated at 7 weeks (Table 6.3).

On 7 week old plants there was some variation in response of cultivars with the different isolates, N2e developing more on Wallace than isolate O4a: however, O4a showed more disease development than N2e on Jet Neuf (Table 6.3). On 13 week plants isolate N2e gave a slightly higher disease rating overall than O4a with no significant interaction with host.

From Figure 6.1, it can be seen that the disease levels on test material of the five seed collections of each cultivar were more variable with plants inoculated after 7 weeks than after 13 weeks. With the

TABLE 6.3: Disease assessment score (0-5) of eight *Brassica* cultivars inoculated with two isolates of *E. cruciferarum* 7 and 13 weeks after sowing.

Plant age (weeks)	Isolate	Cultivar								Mean
		Doon Major	Nevin	Wallace	Bruce	Ruta Otofte	Jet Neuf	Vobra	Rape kale	
7	N2e	2.2	2.0	2.2	1.5	1.5	0.6	1.3	0.4	1.5
	O4a	2.7	2.5	1.6	1.4	1.5	1.2	0.8	0.8	1.6
	Mean	*2.4*	*2.2*	*1.9*	*1.4*	*1.5*	*0.9*	*1.0*	*0.6*	*1.5*
13	N2e	4.5	4.2	4.0	4.2	3.2	3.3	2.9	3.3	3.7
	O4a	4.0	3.8	3.5	3.4	2.8	3.0	2.5	3.0	3.3
	Mean	*4.2*	*4.0*	*3.7*	*3.8*	*3.0*	*3.1*	*2.7*	*3.2*	*3.5*

SED ± (DF = 395)
Host mean 7 weeks = 0.17
" " 13 weeks = 0.16
Isolate mean 7 weeks = 0.09
" " 13 weeks = 0.08

Host x Isolate mean 7 weeks = 0.25
" " " 13 weeks = 0.23

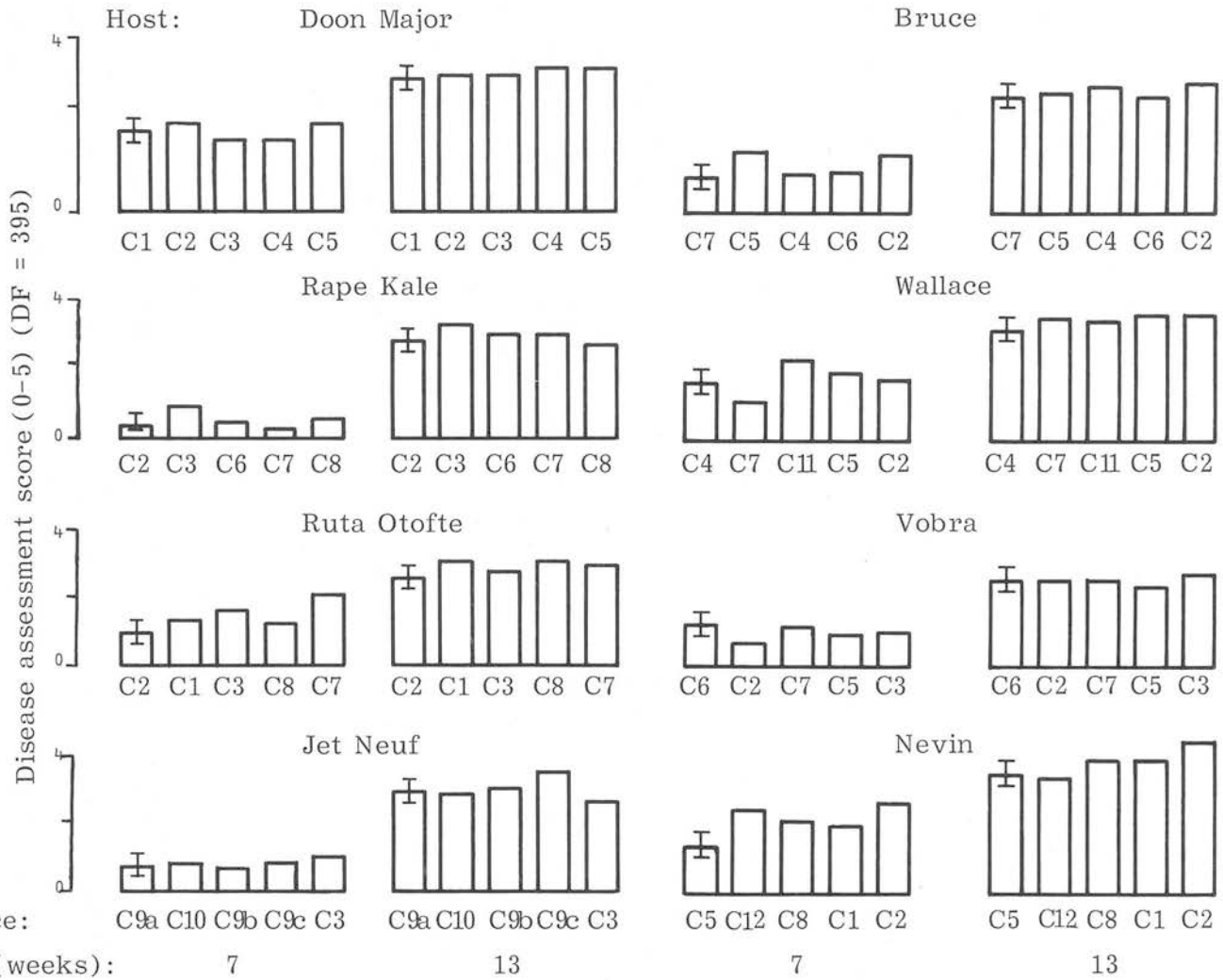


FIGURE 6.1: Disease assessment scores (0-5) of different seed collections of eight *Brassica* cultivars inoculated with *E. cruciferarum* isolates 7 and 13 weeks after sowing.

younger plants significant differences were found between the different seed collections within the cultivars Wallace, Nevin, Bruce and Ruta Otofte. On plants inoculated after 13 weeks only slight, non-significant variations were found between disease levels of plants from different seed collections within cultivars. There was no consistent pattern in disease ratings of plants from seed collections within a cultivar at the two assessment times.

Experiment 6.b: *Mildew development on leaf tissues in relation to plant age*

Mildew development was found to vary with plant age, cultivar and isolate, with significant interactions between plant age and cultivar and between isolate and cultivar. Table 6.4 shows that the average disease index increased with increasing plant age from 5 to 11 weeks, but the disease level was slightly less than the maximum on 12 and 13 week old plants. Doon Major was the most susceptible cultivar with Lunet and Magres intermediate and cultivars Decema and Vobra the least susceptible. On Doon Major, Lunet and Decema the pattern of response to increasing plant age followed the average trend. With Magres, however, there was an increase in susceptibility from 5 to 7 weeks after which the level remained fairly constant: with Vobra, irregular fluctuations were found on plants in relation to age, with little or no positive increase in susceptibility with increasing time.

Overall, isolate N2e gave substantially higher disease ratings than any of the other four isolates (Table 6.5). The mean disease assessment scores were similar for isolates on Decema, but on each of the other hosts the greatest disease levels were found with isolate N2e:

TABLE 6.4: Disease assessment score (0-5) on five *Brassica* cultivars in relation to increasing age.

Cultivar	Plant age (weeks after sowing) 5	7	9	10	11	12	13	Mean
Doon Major	2.6	2.8	3.1	3.5	4.1	3.2	3.9	3.3
Lunet	2.1	2.5	2.7	2.8	3.2	2.2	3.1	2.6
Magres	1.5	2.5	2.3	2.3	2.5	2.2	2.1	2.2
Decema	1.0	1.2	1.1	1.6	1.4	1.2	1.6	1.3
Vobra	1.4	1.0	0.6	1.8	1.9	1.0	1.2	1.1
Mean	1.7	2.0	2.0	2.4	2.6	2.0	2.4	

SED ± (DF = 522)
Plant age mean = 0.11
Cultivar mean = 0.10
Plant age x Cultivar mean = 0.25

TABLE 6.5: Disease assessment score (0-5) of five *E. cruciferarum* isolates on five *Brassica* cultivars.

Cultivar	Isolate N2e	N2d	N3a	O4a	O8	Mean
Doon Major	4.0	3.2	3.6	3.0	2.7	3.3
Lunet	3.2	2.1	2.7	2.6	2.6	2.6
Magres	2.8	1.8	2.2	2.2	2.0	2.2
Decema	1.5	1.1	1.3	1.3	1.3	1.3
Vobra	3.1	0.8	0.9	0.8	0.8	1.1
Mean	2.9	1.8	2.1	2.0	1.9	

SED ± (DF = 522)
Isolate mean = 0.10
Cultivar mean = 0.10
Isolate x Cultivar mean = 0.22

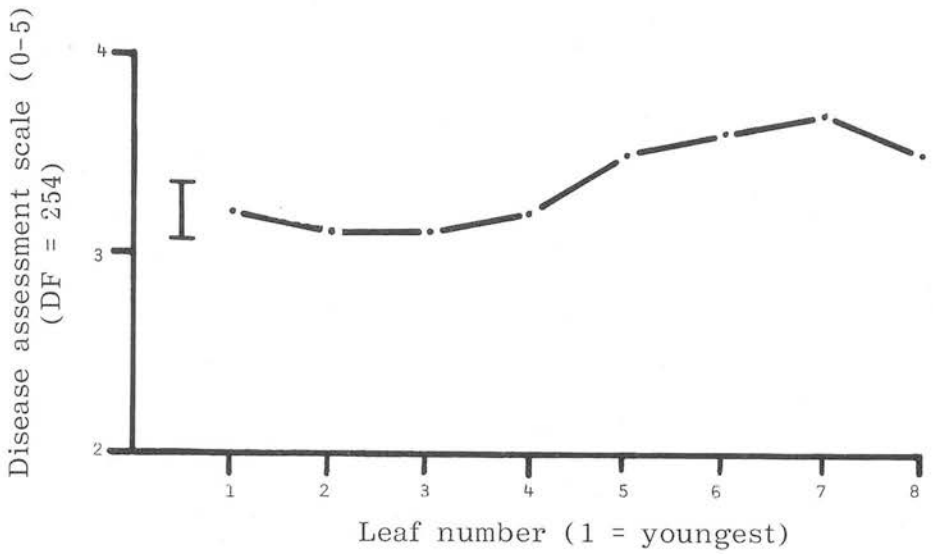


FIGURE 6.2: General pattern of disease levels on leaves of increasing age.

on Vobra, in particular, disease ratings of isolates N3a, N2d, O4a and O8 were low relative to N2e. With the exception of isolate N2e, the other isolates did not vary significantly on any of the hosts.

Experiment 6.c: *Mildew development on leaf tissue in relation to leaf age*

The position of leaves, which reflected their age, in addition to cultivar and isolate, were shown to have a significant influence on disease assessment scores: a significant interaction between cultivar and isolate was also found.

The overall levels of disease averaged for cultivar and isolate, tended to increase with increasing leaf age (Figure 6.2), but differences were small. The four youngest leaves (numbers 1-4) showed generally lower disease scores than leaves 5 to 8.

TABLE 6.6: Disease assessment score (0-5) of eight *Brassica* cultivars inoculated with two isolates of *E. cruciferarum*.

Isolate	Cultivar								Mean
	Doon Major	Bruce	Jet Neuf	Rape Kale	Wallace	Vobra	Ruta Otofte	Nevin	
N2e	4.1	3.6	3.5	3.8	3.4	3.2	3.2	2.6	3.4
O4a	3.3	3.8	3.8	3.4	3.5	3.0	2.7	2.7	3.3
Mean	3.7	3.7	3.6	3.6	3.4	3.1	2.9	2.6	

SED ± (DF = 254)
Cultivar mean = 0.15
Isolate mean = 0.07
Cultivar x Isolate mean = 0.21

Nevin, Ruta Otofte and Vobra showed, on average, less disease development than the other four cultivars, while the mean disease index was larger with isolate N2e than with O4a. However, isolate N2e produced higher disease scores than O4a only on Doon Major, Vobra, Ruta Otofte and Rape Kale, but the disease development found on the other four hosts was slightly greater when infected by isolate O4a (Table 6.6).

Experiment 6.d: *Mildew development on leaf tissues in relation to plant age and pre-inoculation temperature*

Significant effects on disease development were associated with plant age, host and isolate, while the interactions between plant age and temperature and between host and isolate were also significant. Disease levels increased with increasing plant age (Table 6.7). While no significant difference was found on average between plants grown

TABLE 6.7: Mean Disease assessment score (0-5) in relation to plant age and pre-inoculation temperature

Pre-inoculation temperature	Plant age (weeks after sowing) 4	7	10	13	Mean
Cool (9°C)	1.0	1.6	1.7	1.9	1.5
Warm (22°C)	1.7	1.4	1.6	2.0	1.7
Mean	1.4	1.5	1.7	2.0	

SED ± (DF = 493)	Plant age mean	= 0.06
	Temperature mean	= 0.04
	Plant age x Temperature mean	= 0.09

under warm or cool temperatures, there was a difference in response with plants grown under the different temperatures for only 4 weeks before being inoculated: plants grown at higher temperature gave more disease development than plants grown under lower temperature conditions.

In comparing cultivars, on average Doon Major was associated with most mildew, with Lunet and Magres giving rise to slightly less and Vobra least (Table 6.8). Each of the three isolates showed a similar

TABLE 6.8: Disease assessment score (0-5) on four *Brassica* cultivars infected with three *E. cruciferarum* isolates.

	Cultivar				
Isolate	Lunet	Magres	Vobra	Doon Major	Mean
O8	1.8	1.8	0.3	2.2	1.5
N2e	1.7	1.8	1.5	2.2	1.8
O4a	1.9	1.9	0.2	2.2	1.5
Mean	1.8	1.8	0.7	2.2	

SED ± (DF = 493)
Cultivar mean = 0.06
Isolate mean = 0.05
Cultivar x Isolate mean = 0.11

development on the cultivars Lunet, Magres and Doon Major. However, on Vobra isolates O8 and O4a showed very little development whereas isolate N2e progressed almost to the level of that on Lunet and Magres.

Experiment 6.e: *Mildew development on leaf tissue in relation to fertiliser treatment*

The disease levels varied significantly with host but were similar on average for plants fed with the different feeding solutions (Table 6.9). Both isolates tested developed equally well on each host.

TABLE 6.9: Disease assessment score (0-5) and manganese concentration of leaves of five *Brassica* cultivars fed with different feeding solutions, inoculated with isolates of *E. cruciferarum*.

Feeding solution	Cultivars: Doon Major	Vobra	Lunet	Magres	Decema	Mean
	*Disease assessment score**					
Solufeed	4.7	3.7	2.8	3.0	1.3	3.1
Sangral	4.7	3.3	3.5	2.8	1.4	3.1
Mean	*4.7*	*3.5*	*3.1*	*2.9*	*1.3*	
	Manganese conc (ppm DM)					
Solufeed	121	129	140	120	160	134
Sangral	116	100	123	140	116	119
Mean	*118*	*114*	*131*	*130*	*138*	

* SED ± (DF = 95)
Cultivar mean = 0.26
Feeding solution mean = 0.16
Cultivar x Feeding solution mean = 0.36

Doon Major showed most disease development: cultivars Vobra, Magres and Lunet produced moderate levels and Decema exhibited only slight infection. The overall manganese concentration was higher with cultivars fed with Solufeed than with Sangral.

Experiment 6.f: *Influence of leaf type on host susceptibility to mildew infection*

With both 7 and 13 week plants (Table 6.10), Doon Major showed much more mildew development than any other host and A4207 plants with normal leaves gave higher scores than those with glossy leaves. With 13 week old plants, normal A8022 plants gave more infection than those of the same line with glossy leaves. Glossy and normal plants of breeding lines A8022 and A4207 were easily identified. However, plants classified as of different leaf types, of breeding line A8024 were less easily distinguished: at 7 and 13 weeks it was not possible to detect any difference between plants previously categorised into the two groups, and their mildew levels were similar.

On 7 week old plants the development of N4a was on average less than that of isolates N2e and O4a.

TABLE 6.10: Disease assessment score (0-5) in relation to breeding line and leaf type of Brussels sprout.

Breeding line	Leaf type	Plant age (weeks) 7	13
A4207	Glossy	0.9	0.9
	Normal	1.4	1.4
A8022	Glossy	1.6	1.3
	Normal	1.7	2.8
A8024	Glossy	1.2	1.1
	Normal	1.3	1.4
Doon Major (susceptible control)		4.9	5.0
	SED ± (DF = 100)	0.16	0.18

Experiment 6.g: *Effect of different leaf washing treatments on cultivar susceptibility*

With both 7 and 13 week old plants, significant effects of host, isolate and washing treatment on mildew development on leaf discs were observed, and there were significant interactions between host and isolate.

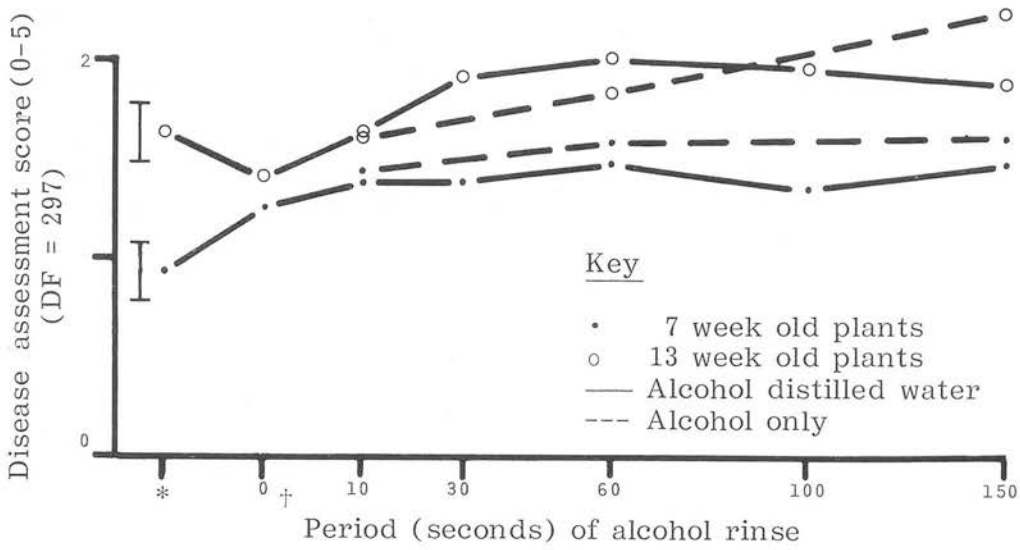
In general, all hosts were more susceptible 13 weeks after sowing than at 7 weeks (Table 6.11). The ranking of cultivars in order of disease development was more or less similar at both plant ages, with

TABLE 6.11: Disease assessment score (0-5) with different *Brassica* hosts inoculated with *E. cruciferarum* isolates 7 and 13 weeks after sowing.

Isolate	Plant age (weeks after sowing)	Cultivar					Mean
		Doon Major	Vobra	Achilles	Barsica	BC82	
N4a	7	3.6	1.6	1.0	0	0	1.2
O4a	7	2.5	2.0	1.1	1.9	0	1.5
	Mean	*3.1*	*1.8*	*1.0*	*0.9*	*0*	*1.35*
N2e	13	3.0	3.4	1.1	1.7	0.4	1.9
O4a	13	2.7	2.6	1.1	1.7	0.2	1.7
	Mean	*2.9*	*3.0*	*1.1*	*1.7*	*0.3*	*1.8*

SED ± (DF = 297)	Cultivar mean	=	0.01 (*7 weeks*)	0.11 (*13 weeks*)
	Isolate mean	=	0.07	0.07
	Cultivar x Isolate mean	=	0.16	0.15

Doon Major and Vobra proving most susceptible, Achilles and Barsica intermediate and BC82 highly resistant. BC82 did not show any disease development on leaf discs from 7 week old plants regardless of the treat-



* no treatment
† water only

FIGURE 6.3: Disease development on leaf discs from 7 and 13 week old plants following treatments with alcohol (70%) and water.

ment and, after 13 weeks only slight disease development was found. Isolate N4a did not develop beyond the appressorial stage on leaf discs of 7 week old Barsica plants but isolate O4a developed moderately well: with 13 week Vobra plants isolate N2e developed more than isolate O4a.

From Figure 6.3 it can be seen that on leaf discs from plants at both 7 and 13 weeks the treatment of leaves with alcohol prior to inoculation tended to increase disease development from the levels on the untreated leaf discs. With 7 week plants disease development was also lower on leaf discs which received no treatment than on leaf discs treated with distilled water only but with 13 week old plants, disease development was greater on leaf discs which received no treatment than on discs treated with distilled water. Although with 7 week old plants there was no significant difference between disease on discs treated with water only and that on discs treated with alcohol and water,

the level of disease was always greater on discs which received the latter treatment. There was no significant interaction between leaf treatment and cultivar but the greatest responses to alcohol treatment of leaves were found with Vobra (Figure 6.4).

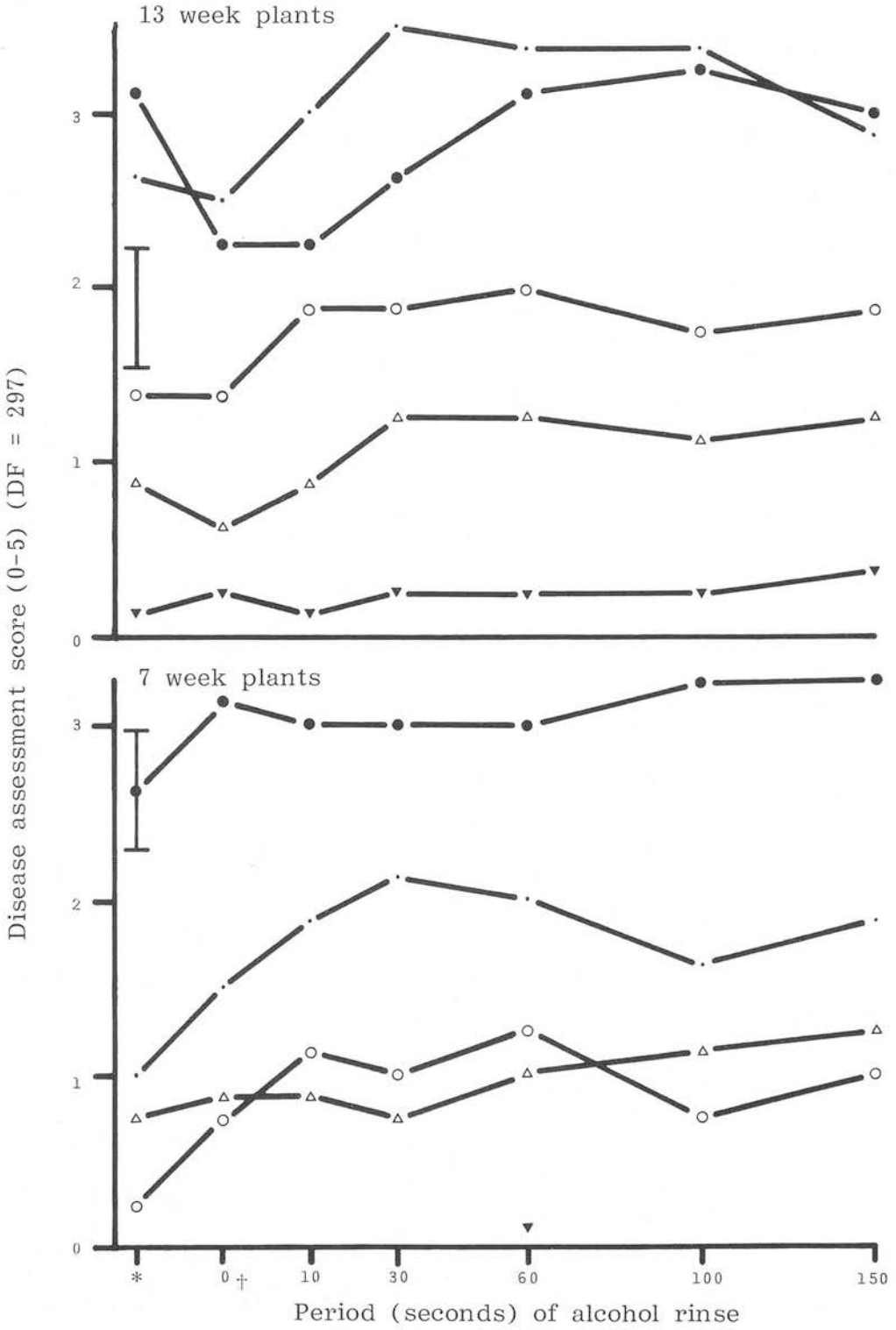


FIGURE 6.4: Disease assessment score (0-5) for hosts of different ages inoculated with *E. cruciferarum* isolates following leaf washing treatments.

Key: Hosts

- ● Doon Major
- · Vobra
- △ Achilles
- ○ Barsica
- ▼ BC82
- * no treatment
- † water only

DISCUSSION

Brassica campestris is an outbreeder with a sporophytic incompatibility system (McNaughton, 1976); *B. napus* is predominantly self-fertilised (Josefsson, 1948) but there is little doubt that some crossing occurs under natural conditions (Palmer, 1962). Consequently, variability within and between different seed populations of each cultivar of *B. napus* and *B. campestris* is to be expected. In testing intra-cultivar variation between plants from different seed sources, only four of the eight cultivars used showed significant variation at the 7 week stage and none at the 13 week stage. Variation associated with cultivar or with stage of growth was always greater than that occurring within cultivars. While intra-cultivar variation is liable to occur, it does not appear to be of a high magnitude and generally each seed collection of a cultivar tended to reflect average cultivar characteristics and was rarely outside the normal range of variation.

In Experiment 6.f where glossy variants were compared with normal leaf types of breeding lines, the extent of growth of *E. cruciferarum* on the glossy variants of two lines was found to be reduced on 7 and 13 week old plants. The reason for the reduced growth on the glossy variants is not known, but several factors may be involved. Glossy variants differed from normal plants in the extent of cuticular wax formation (Wills, pers. comm.). The effect of the leaf surface characteristics on growth of powdery mildew fungi has been the subject of much debate. Numerous experiments have shown that germination may be enhanced on the leaf surface as opposed to glass surfaces (Peries, 1962a; Schnathorst, 1960). The treatment of glass surfaces with leaf surface waxes has also given rise to greater germination percentages

on the treated surfaces (Peries, 1962b). Leaf surface wax appears therefore, to have an influence on the establishment of a pathogen/host interaction with, perhaps, a constituent of the wax or a physical characteristic providing a necessary stimulus for further development. Such a stimulus may be lacking or in reduced amounts on the glossy leaf types, with the result that disease development is delayed. The hydrophobic property of leaf wax affects the retention of water to the leaf surface and, since glossy plants have less cuticular wax (Wills, pers. comm.), water may be retained more readily and conidial germination and growth consequently delayed (Wheeler, 1981). In 1971, Purnell carried out leaf washing experiments, and found that washing prior to inoculation with *E. cruciferarum* conidia caused a reduction in hyphal growth. He concluded that both inhibitory and stimulatory substances are present on swede leaves, the inhibitors being produced on the leaf surface much more slowly than the stimulatory substances. In 1971, Blakeman reported that wax constituents may restrict the movement of leaf exudates. Perhaps, therefore, the increased disease development on the normal leaf types was due to the wax layer acting as a barrier to the exudation of the inhibitory substances, whereas the reduced cuticular wax on glossy types was a less effective barrier to exudation of inhibitory substances and consequently disease development was inhibited.

From leaf washing experiments with *B. napus* and *E. cruciferarum*, Purnell (1971) concluded that inhibitory substances were present at the leaf surface. More recent work (Khairi and Preece, 1979) demonstrated that washing uninfected leaves subsequently allows greater development of powdery mildew fungi. The results of Experiment 6.e, for both 7 and 13 week old plants, with only one exception, agree with these earlier

findings, and implicate the presence of an inhibitory substance at the leaf surface which is removed by the washing treatment. In these studies, an exception occurred with 13 week old plants, whereby the water only treatment reduced the susceptibility of the cruciferous hosts tested. This response may be explained by a differential rate of nutrient leaching which occurs between young and old plants, young actively growing tissue showing relatively little loss of carbohydrates, while more mature tissue is very susceptible to leaching (Tukey, 1971). In 1971, Purnell also observed a reduction in hyphal growth of *E. cruciferarum* on washed swede leaves and attributed this to an impoverishment in the nutrient status of the leaf surface and more particularly, to loss of carbohydrates. Stimulation of facultative parasites by nutrients on the leaf surface is a well recorded phenomenon. However, with obligate parasites such as powdery mildews, which have been shown to obtain their nutrients from the host via haustoria (Bushnell and Gay, 1978), stimulation of growth by leaf surface nutrients is a novel conception. Wheeler (1981) suggested that nutrient absorption through haustoria is supplemented by direct absorption by the hyphae on leaf surfaces. As there are many examples of the inhibition of obligate parasites by substances on the leaf surface (Preece and Dickinson, 1971), it would appear likely that stimulation could also occur. Moreover, Bushnell and Gay (1978) observed that after removal of the haustoria from mildew colonies, mycelial growth stopped immediately but resumed a few hours later and grew slowly for the next 24 to 36 hours. In addition, they noted that growth was better with than without glucose or sucrose applied to the leaf surface. They concluded that small amounts of solutes from the host cells possibly entered hyphae directly through the leaf surface but nearly all nutrients were transferred via haustoria.

The relationship between age of tissues and susceptibility varies with different pathogen/host combinations. The resistance of cereals to *E. graminis* is reported to increase with plant age (Large and Doling, 1962) whereas tobacco leaves become more susceptible to *E. cichoracearum* as they mature (Cole, 1966). Several workers (Brain, 1978; Munro, Dickinson and Scourey, 1981) found that powdery mildew (*E. cruciferarum*) of swedes was more severe the earlier cultivars were sown. Furthermore, during field trials, Brain (1978) observed that young leaves were more resistant than mature leaves. Although this implies that swede cultivars become more susceptible with increasing age, factors such as density of the crop canopy and temperature will have influenced the levels of infection. Artificial inoculation experiments with leaf discs of two swede cultivars incubated under controlled conditions by Brain (1978) revealed, in contrast with his field observations on the same cultivars, that resistance of leaves increased with increasing physiological age. The experimental methods used in these studies were very similar to those of Brain (1978) in his inoculation studies with leaf discs, but the results show resistance decreasing with increasing plant age and leaf age. If, as Brain suggests, resistance increases with leaf age the more mature plants become the greater will be the area of increasingly resistant leaf tissue which would therefore slow down mildew development. Consequently, the field trials results (Doling and Willey, 1969; Munro *et al.*, 1981) would not be expected to demonstrate, as they do, that later sown plants are less affected by mildew. The conclusion from the present studies, that susceptibility increases with plant or leaf age, is in keeping with the results from field trials (Doling and Willey, 1969; Munro *et al.*, 1981).

In each of the experiments where plants 7 and 13 weeks of age were inoculated, susceptibility increased with age of host tissue. The decrease in susceptibility of 13 week old plants after washing with water was considered to be due to the removal of a stimulatory substance from the leaf surface. The exudation of nutrients onto older leaves (Tukey, 1971), however, does not wholly account for the increase in susceptibility with age since, on removal of the substance by the water treatment, susceptibility did not fall to the levels found on younger leaves. The greater resistance of less mature tissue may reflect other factors. Hwang and Heitefuss (1982) considered that changes in resistance with age merely reflected morphological and physiological differences in tissues. Decreased resistance may be a function of the accessibility of nutrients from the host tissue, i.e. during the period of rapid leaf growth, the pathogen competes less effectively for metabolic products (Cole, 1966) and the leaching of nutrients to the leaf surface is minimal (Tukey, 1971). This may explain adult plant resistance of cereals, in which resistance of a leaf increases to a maximum when it is fully expanded, after which susceptibility increases until senescence (Jones and Hayes, 1971). Moreover, the functioning of active defence mechanisms may change with age.

The variability of resistance has been studied in several diseases and such environmental factors as light, moisture, nutrition, and temperature have been shown to alter not only the degree of resistance but also the type of symptoms which develop. For example, the wheat cultivar Marquis is resistant to race 139 of *Puccinia graminis* var. *tritici* below 24°C but is susceptible at 30°C (Stakman and Harrar, 1957). In 1951, Tapke revealed that the environmental conditions to which plants are exposed before inoculation may greatly influence their reaction

to mildew. For example, susceptibility was enhanced by pre-inoculation conditions such as higher temperatures and increased watering; conversely, resistance was promoted by exposure to cool pre-inoculation temperatures. Predisposition by high or low temperatures has been demonstrated with many pathogen/host combinations (Colhoun, 1973). In Experiment 6.d, with one exception, there was no difference in susceptibility of either cultivar when grown under cool (9°C) or warm (22°C) temperatures.

Numerous experiments have shown that fertiliser applications to plants can dramatically affect their susceptibility to disease (Tarr, 1972). In 1981, Brain and Whittingdon found a positive correlation between increasing leaf manganese concentration and decreasing susceptibility of swede cultivars to *E. cruciferarum*. From an experiment (6e) carried out to compare the influence of two feeding solutions on host susceptibility, it was concluded that they had little effect (Table 6.9). However, although tissue manganese concentrations were variable, there was a general tendency for susceptibility to decrease with increasing leaf manganese concentration, in accordance with the findings of Brain and Whittingdon (1981). The precise role manganese plays in increasing resistance of tissues to mildew is not known, but has been observed to reduce fungal growth (Cochrane, 1958). Manganese is an essential trace element for plant growth and acts as a catalyst in several important enzymatic and physiological reactions in plants (Sauchelli, 1969), some of which may be involved in host resistance (Heitefuss and Williams, 1976).

7. GENERAL CONCLUSIONS

In inoculation tests on the host range of *E. cruciferarum* only members of the families Cruciferae and Papaveraceae were infected; no infection occurred on plants selected from the families Chenopodiaceae, Compositae, Cucurbitaceae, Fumariaceae, Geraniaceae, Graminae and Leguminosae. Various cruciferous weed species showed a variety of responses in the infection studies: species which proved highly susceptible included *Sinapsis arvensis* and *Sisymbrium officinale*, those showing moderate resistance included *Thlaspi arvense*, *Lepidium sativum*, *Cardaria draba* and *Capsella bursa-pastoris*, while those which were highly resistant included *Arabidopsis thaliana* and *Raphanus raphanistrum*. From tests with different isolates of *E. cruciferarum* collected from different hosts and sources on a comprehensive range of cultivated cruciferous hosts, including members of *Brassica campestris*, *B. carinata*, *B. juncea*, *B. napus*, *B. oleracea*, *Raphanobrassica*, *Raphanus sativus* and *Sinapsis alba*, differences in disease levels following inoculation were evident both between and within species. Species exhibiting high levels of resistance included *B. carinata*, *S. alba*, *Raphanobrassica*, *R. sativus* and *B. juncea*. The swede cultivar Doon Major (*B. napus*) proved to be a universally susceptible host which always showed high disease levels relative to the other cultivars or lines tested.

Most isolates were able to infect, at least to some degree, most cruciferous hosts and cross-infections between isolates of *E. cruciferarum* from different host species or genera readily occurred. The level of resistance or susceptibility of particular hosts tended to be reflected over the whole range of isolates, although some variation in the overall level of disease development associated with particular isolates occurred. Variation among isolates, however, was generally quantitative rather than qualitative, with only a few instances of clearly defined host

specificity. Isolates collected from *B. napus* and *B. oleracea* had a slightly greater general affinity for the respective original host species. Specific interactions with large effects occurred only occasionally within host species at a cultivar level, notably the activity of an isolate of *E. cruciferarum* originally from *B. napus* to cause advanced levels of infection on the *B. napus* cultivar Barsica and the ability of an isolate from *B. oleracea* to cause extensive infection on Cluseed Early (*B. oleracea*).

In comparing the behaviour of different isolates on the susceptible cultivar Doon Major (*B. napus*), all showed progressive development and produced conidiophores by 96 hours. Colony development of different isolates varied with time but variation in patterns of growth between isolates was inconsistent and did not suggest large intrinsic differences occurred within the fungal species. Conidia were produced from about 4 days after inoculation and the maximum daily rate of conidia production occurred at about 13-14 days. There did not seem, however, to be an obvious relationship between the rate of colony development and the level of early production of conidia. The sporulation capacity of colonies was inversely related to inoculum density. The effect of inoculum density on conidial production was seen from 9 days after inoculation. The length and breadth of conidia varied only slightly between isolates: the mean length ranged from 42 to 45 µm and the mean breadth from 14 to 20 µm.

Observations on fungal development of different isolates of *E. cruciferarum* on different cruciferous hosts which included members of *B. campestris*, *B. carinata*, *B. napus*, *B. oleracea*, *R. sativus* and *Raphanobrassica* showed that rates of germ tube production and appressorium formation were similar for all pathogen/host combinations. Fungal

development was affected at various stages after appressorium formation on the more resistant hosts Vobra (*B. campestris*), BC82 (*B. carinata*), Barsica (*B. napus*), Achilles and Cluseed Early (*B. oleracea*), RS15 (*R. sativus*) and RB25/8 (*Raphanobrassica*). Compared with Doon Major, all hosts tested exhibited a degree of resistance expressed at a complete or partial level. Complete resistance prevented growth beyond the appressorial stage, although it was never found to occur with all infection units. Infection of this type prevented the establishment of primary infection and reduced the number of colonies which developed from a standard level of inoculum. Partial resistance emerged in this study as causing a delay or restriction in the progressive development of colonies and a reduced sporulation capacity of colonies: consequently, the rate of pathogen spread would be reduced.

A successful parasitic relationship between the powdery mildew fungi and their hosts is dependant upon the establishment of functional haustoria. Studies on the development of haustoria with different isolate/host combinations showed that the rate of haustorial development varied with degree of host susceptibility. Haustoria were produced more rapidly and more extensively on the susceptible cultivar Doon Major than on other hosts showing some level of resistance. More resistant hosts showed greater numbers of abnormal haustoria. Resistance was therefore associated with both reduced haustorial numbers and reduced haustorial efficiency. Various tissue responses to infection by *E. cruciferarum* were observed. Cell necrosis was recorded relatively early after inoculation on more resistant hosts and was associated with restricted infection: with a greater level of host resistance smaller numbers of necrotic cells were observed. In considering further the relationship between cell necrosis and fungus-plant cell compatibility,

a negative correlation was found between the frequency of successful penetrations and frequency of host cell necrosis. On the susceptible cultivar Doon Major, extensive cell necrosis, which occurred late in the infection cycle (144 hours after inoculation), was related to cell exhaustion at an advanced stage of infection as a consequence of the demands made by the fungus.

Callose deposition occurred in epidermal cells of all hosts as a generalised response to penetration by *E. cruciferarum*. From transverse section studies callose deposition in response to penetration was localised around penetration hyphae and directly above haustoria. On the more resistant hosts callose deposition was found to continue from the initial apposition, progressively encapsulating haustoria and thus providing a defence response to infection by *E. cruciferarum*. Lignified materials were detected in appositions and lateral walls but no relationship between lignification and resistance was confirmed. Phenolic compounds were not detected at sites of penetration.

Although *B. campestris* and *B. napus* have outbreeding characteristics, intra-cultivar variation was not of a high magnitude, seed collections of a cultivar from different sources tending to reflect average cultivar characteristics. However, leaf surface characteristics affected host resistance to *E. cruciferarum* infection. Variants of *B. oleracea* with reduced cuticular wax were more resistant. Washing leaves of 7 week old plants with water gave increased infection levels, whereas washing of 13 week old plants reduced infection. Washing leaves in alcohol prior to inoculation invariably increased infection levels. These various responses to washing of plants of different ages may be related to changes in the presence of either inhibitory or stimulatory substances at the leaf surface. Host susceptibility tended to increase with plant

or leaf age and this may be again linked to changes in leaf surface characteristics. Pre-inoculation growth temperature of plants and type of commercial fertiliser used did not influence the infection response of plants but there was evidence that increasing manganese content of leaves slightly increased their resistance.

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